EVALUATING THE EFFICACY OF EXOGENOUS ENZYMES ON NUTRIENT DIGESTIBILITY AND BROILER PERFORMANCE

A Dissertation

by

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ABSTRACT

The objective of this research was to evaluate the efficacy of exogenous enzymes on nutrient digestibility and broiler performance. Experiment one evaluated the addition of a corn-screenings in low-energy corn-soybean meal diets with and without NSPase supplementation. The addition of corn-screenings hindered growth performance with reductions in feed consumption, body weight, and an increase in FCR. Removing energy in the diet increased FCR throughout the study. The inclusion of NSPase reduced FCR throughout the trial compared to the control. These results demonstrate the effectiveness of NSPase inclusion on growth performance in reduced energy diets containing lower quality feed ingredients.

Experiment two consisted of three studies evaluating the inclusion of a cocktail NSPase in low-energy diets on broiler growth performance and processing. Each study consisted of three dietary treatments including a positive control (PC), negative control (NC) with a 132 kcal/kg reduction in energy, and NC with NSPase supplementation. Broiler performance was improved in each study with the inclusion of a cocktail NSPase increasing body weight and improving FCR. Furthermore, supplementing NSPase increased multiple processing parameters when compared to the reduced-energy diet. The results of this experiment confirm the ability of exogenous enzymes to improve broiler performance and processing parameters when included in reduced-energy diets.

Experiment three determined the impact of increasing levels of phytase inclusion on nutrient digestibility, bone mineralization, and broiler performance. Reducing

phosphorus levels in the NC negatively influenced bone mineralization, nutrient digestibility, and growth performance throughout the experiment. The supplementation of increasing doses of phytase linearly and quadratically improved all parameters evaluated when included in low-phosphorus broiler diets. Additionally, the supplementation of super-doses (> 1,500 FTU/kg) of phytase yielded more pronounced benefits when compared to phytase included at conventional levels justifying the potential use of greater concentrations of phytase in broiler diets. The results of this experiment suggest that greater levels of phytase may elicit a more pronounced improvement on nutrient digestibility, bone mineralization, and growth performance compared to conventional doses of phytase.

DEDICATION

This dissertation is dedicated to my family and all those that have influenced my life both professionally and personally by continuing to challenge me to strive for excellence and to never accept mediocracy.

To my mom, thank you for always supporting me through the good, the bad, and the ugly times and for always being there to pick me up when I had fallen. You showed me how to put my faith and trust in God and taught me the importance of family and love. Words cannot describe how grateful I am for all that you have done for me. You have always been my biggest fan and I cannot thank you enough for instilling in me the character and morals that I have today. You will always be an inspiration to me and I love you to the moon and back.

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NOMENCLATURE

ADF	Acid detergent fiber
AME	Apparent metabolizable energy
AOAC	Association of Agriculture Chemists
BW	Body weight
DDGS	Distillers dried drains with solubles
FC	Feed consumption (gram/bird/day)
FCR	Feed conversion ratio
FTU	Phytase unit
IACUC	Institutional Animal Care and Use Committee
IDE	Ileal digestible energy
ME	Metabolizable energy
NC	Negative control
NDF	Neutral detergent fiber
NPP	Non-phytate phosphorus
NSP	Non-starch polysaccharides
NSPASE	Non-starch polysaccharide degrading enzymes
PC	Positive control
WOG	Without Giblets

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION

Feed costs account for approximately 70 to 75% of total production expenses in the poultry industry. Although corn and soybean meal are the primary constituents in U.S. poultry feeds, greater incorporation of alternative feedstuffs and by-product ingredients have gained increasing attention in an effort to minimize feed costs. Although these lower-quality ingredients provide a cost-savings opportunity for producers, several factors limit higher inclusion rates in poultry feeds (Van Immerseel et al., 2017). The inclusion of exogenous enzymes in poultry feeds has been utilized for the past several decades to allow for greater nutrient digestibility, increased formulation flexibility, higher inclusions of lower-quality ingredients, and reductions in feed costs without sacrificing bird performance (Choct, 2006; Ravindran, 2013).

Numerous research papers have been published evaluating the addition of non-starch polysaccharide degrading enzymes (NSPase) such as xylanase and enzyme cocktails which contain numerous enzymes (β-mannanase, α-galactosidase, xylanase, amylase, mannanase, cellulase, pectinases etc.) on nutrient digestibility and broiler performance. The inclusion of NSPase employs several mechanisms that hydrolyze the indigestible bonds in the cell walls of plants, thus improving nutrient digestibility and animal performance (O'Neill et al., 2014; Slominski, 2011). However, due to the increasing variability and nutrient composition of poorer quality feedstuffs such as

DDGS and cereal grain by-products, further research is required to determine the impact of NSPase in diets containing varying levels of lower-quality ingredients.

The addition of phytase in poultry diets has been an advantageous strategy implemented by poultry producers in order to enhance phosphorus utilization, increase bird performance, and limit nutrient excretion (Selle and Ravindran, 2007). While lower doses (< 500 FTU/kg) of phytase has been traditionally supplemented to improve nutrient availability and increase broiler growth, strategies involving super-dosing phytase have become an increasing opportunity for poultry integrators. Inclusion of phytase at levels beyond 1,500 FTU/kg have suggested further improvements in both nutrient utilization as well as growth performance compared to lower doses of phytase (Augspurger and Baker, 2004; Pirgozliev et al., 2011; Shirley and Edwards, 2003). While this may be an effective strategy for producers, current research involving the impact of super-dosing phytase on nutrient digestibility and growth performance is rather limited but necessary to evaluate ideal phytase inclusion levels in corn-soy diets and the mechanism behind the improvement in growth performance.

The goal of the research described herein was to assess the impact of NSPase in poultry diets with varying concentrations of lower-quality ingredients as well as evaluating the effect of increasing levels of phytase to determine optimum enzyme inclusion as a strategy to reduce production costs through enhanced nutrient utilization and growth performance.

CHAPTER II

LITERATURE REVIEW

Use of By-product and Alternative Ingredients

Increasing development of viable, renewable energy sources accompanied with an on-going demand to sustain the world's rising human population, has re-directed a vast majority of crop production towards human consumption. It has been suggested that by 2050 the world's crop production must increase by 100 to 110% in order to withstand the global demand for food and energy (Tilman et al., 2011). Furthermore, the continuous depletion of finite fossil fuel resources and environmental impact concerns has led to the increase in biofuel production, thus further increasing the demand for crop production. Renewable biofuel production in the United States (U.S.), predominantly in the form of ethanol, is grain-based with over 95% of the ethanol produced being derived from corn (Simpson, 2009). Currently, the U.S. is the number one producer of ethanol with a near four-fold increase in production over the last decade (Havlin, 2018). This increase has led to approximately 40% of the U.S. corn crop being designated for ethanol production with the remainder being used for livestock feed (USDA, 2012). As the production of biofuels intensifies, supplies of feed ingredients become tighter, thus placing more pressure on the livestock industry through higher ingredient prices (Baier, 2009; Rosentrater, 2011). In order to mitigate the impact of rising ingredient costs, the incorporation of alternative and by-product ingredients have become an increasingly

common practice for livestock and poultry producers looking to decrease feed costs (Van Wyhe et al., 2012).

The most common animal by-products used in broiler diets include meat and bone meal, blood meal, feather meal, and poultry offal/by-product meal (Caires, 2010). These feed ingredients are typically recovered after slaughter and are further processed through rendering methods making them adequate for feed use (Kim et al., 2012b). Once rendered, these animal by-products are considered ideal protein sources with a wide source of available amino acids, as well as contributions to vitamins and minerals (Caires, 2010; Jayathilakan et al., 2012). Wang and Parsons (1998) reported the average amino acid content from thirty-two meat and bone meal samples being 53.2% CP, 2.73% Lys, 0.6% Cys, and 0.75% Met on a DM basis. Furthermore, Han and Parsons (1990) identified crude protein and amino acid levels for both feather meal (86.25% CP, 2.02% Lys, 4.52% Cys, and 0.40% Met) and poultry by-product meal (58.10% CP, 2.94% Lys, 1.29% Cys, and 1.10% Met) using true digestibility assays. While the average inclusion of animal based protein feedstuffs in U.S. broiler diets is approximately 3% (Waldroup, 2002), no impacts on broiler performance have been observed when including meat and bone meal at levels up to 5% (M. Bozkurt, 2004). Although these animal by-products provide a more complete amino acid profile compared to plant-based proteins (Donadelli et al., 2018), rendering methods including pressurization (Shirley and Parsons, 2000), prolonged drying times and overheating (Kim et al., 2012b; Papadopoulos et al., 1985), as well as batch to batch nutritional variability (Wang and Parsons, 1998) can lead to inconsistent nutrient composition, thus limiting higher inclusion rates.

Plant derived by-products provide additional cost-savings opportunities with the majority of by-products originating from the further processing of cereal grains. In monogastric diets such as swine and poultry, cereal grains are the primary energy components, and therefore represent the highest cost among ingredients due to their substantial inclusion rates (Agudelo Trujillo, 2009). In the U.S., corn is the main cereal grain used for the production of biofuels or human food through wet milling, dry milling, or dry grinding processes (Kim and Dale, 2004; Rojas et al., 2013). These manufacturing methods yield corn by-product ingredients such as corn screenings (by-product obtained from the cleaning and screening of whole corn) (Meinders, 1993), corn gluten meal, corn germ meal, and corn-gluten feed as a result of the wet-milling process and dried distiller's grain with solubles (DDGS) produced from dry-milling (Rojas et al., 2013; Stein et al., 2016). Of these by-products produced, DDGS is the most popular as it is the primary by-product derived from ethanol production, thus making it readily available and relatively low cost (RFA, 2008; Rosentrater, 2011).

One concern with incorporating DDGS in poultry diets in large amounts is composition. Depending on the ethanol plant, some or all solubles produced via evaporation of thin stillage are added back to the wet grains and dried. This process results in greater concentrations of fat and ash in the final by-product (Bregendahl, 2008; Noll 2007). It has been suggested that every bushel of corn (56 lbs) yields approximately 2.9 gallons of ethanol, 18 lbs of carbon dioxide, and 18 lbs of DDGS (Rosentrater, 2011). During this fermentation process, grain starch within the corn's endosperm is converted to ethyl alcohol and carbon dioxide, increasing the concentration of the

residual nutrients by 2 to 3 times (Bregendahl, 2008). As a result, DDGS provide an exceptional source of crude protein, amino acids, phosphorus (P), and other nutrients (Swiatkiewicz et al., 2016). Batal and Dale (2006) reported the nutrient composition of 17 DDGS samples with values ranging from 23.0% to 30.0% for crude protein (CP), 2.5% to 10.6% for crude fat, 5.1% to 8.1% for crude fiber, 3.9% to 5.4% for ash, and an average metabolizable energy (TME_n) value of 2820 kcal/kg. Furthermore, Noll et al. (2003) analyzed 20 corn DDGS samples from five commercial ethanol plants with mean values for CP, crude fat, crude fiber, and ash being 27.6, 10.0, 5.7, and 4.0%, respectively.

Although DDGS possess higher concentrations of nutrients as a result of starch fermentation, greater non-starch polysaccharide (NSP) levels (consequence of higher fiber content) accompanied with inconsistent nutrient quality and composition warrants concern when included in poultry diets (Cromwell et al., 1993; Knudsen, 1997; Swiatkiewicz et al., 2016). Corn is typically composed of approximately 8.1% NSP compared to NSP levels of 25.0% in corn DDGS (Jaworski et al., 2015). It is known that the anti-nutritive properties associated with NSP can negatively influence nutrient digestibility and absorption resulting in an adverse effect on broiler performance (Slominski, 2011). Additionally, fermentation and drying processes can alter NSP characteristics (Widyaratne et al., 2009) as well as negatively impact amino acid content and digestibility (Almeida et al., 2013; Bregendahl, 2008). Although CP levels in DDGS are higher than that of whole corn, amino acid digestibility tends to be lower depending on the drying process utilized by the manufacturer (Bregendahl, 2008; Waldroup et al.,

2007). Drying procedures and practices can adversely affect amino acid availability with excess heat being most detrimental to lysine (Almeida et al., 2013). Although the digestibility of various amino acids is impacted, lysine digestibility seems to be the lowest and most variable among essential amino acids (Cromwell et al., 1993; Waldroup et al., 2007). In a trial conducted by Adedokun et al. (2015) evaluating the standardized ileal amino acid digestibility (SIAAD) of DDGS and corn when fed to broilers, the mean SIADD of lysine from five DDGS samples was substantially lower at 58.32% compared to a mean SIADD of 92.4% from corn. Additionally, Kim et al. (2012a) reported a wide variation in total lysine content as well as digestibility among samples with dietary lysine content ranging from 0.67 to 1.01%. Variations in dietary amino acid content and digestibility due to differences among manufacturing processes, accompanied with higher concentrations of NSP, limit higher inclusions of DDGS and must be considered when evaluating DDGS inclusion levels in broiler diets.

Due to the variations in nutrient content in DDGS as discussed above, DDGS has usually been included at levels of 5% or less in commercial poultry diets in the past (Lumpkins et al., 2004). However, recent studies indicate higher levels may be feasible without adverse effects on broiler performance. In a series of experiments conducted by Lumpkins et al. (2004), birds were fed increasing levels of DDGS (ranging from 0 to 18%) in corn-soybean diets and formulated based on a total amino acid basis. As a result, Lumpkins et al. (2004) stated that DDGS could be included at 6% in the starter phase and increased to 12 and 15% in the grower and finisher periods, respectively. Similarly, Wang et al. (2007) conducted a trial evaluating increasing levels of DDGS (0,

15, and 30%), and reported no differences in live performance or carcass characteristics with 15% inclusion of DDGS. However, the addition of DDGS at 30% reduced body weight (BW) and feed consumption (FC) while negatively affecting breast meat yield. Furthermore, Loar et al. (2012) reported that birds fed 8% DDGS through 28 days were significantly lighter in BW and exhibited a higher feed conversion ratio (FCR). These series of experiments indicate that DDGS may be a viable ingredient for use in poultry diets, however, bird age and DDGS inclusion level must be considered as to not negatively impact performance associated with ingredient variability or NSP anti-nutritive properties.

Despite the fact that DDGS is one of the major by-products utilized by poultry producers, the incorporation of corn screenings in broiler diets may also be a potential by-product for dietary inclusion. Corn screenings are a by-product obtained from the handling and repetitive screening of corn prior to exportation (Hess et al., 1999). The generation of screenings generally occurs throughout the handling and processing of whole corn in which screenings typically consist of broken kernels, weed seeds, cob and other foreign non-grain material (Bern, 1992; Hurburgh, 1995; Lin and Lin, 1994). The majority of corn screenings are sold as by-product feeds with an approximate value being 70 to 80% of the price of corn (Lin and Lin, 1994). The lower ingredient costs associated with screenings can be attributed to the lower nutrient values compared to that of whole corn, as well as the wide variation in nutrient levels and presence of mycotoxins and anti-nutritive factors such as NSP (Hess et al., 1999; Lin and Lin, 1994). When the corn kernel is broken and the endosperm is exposed, such as that in corn

screenings, the probability of fungal growth is increased, thus making screenings an exceptional media for mycotoxin production such as fumonisin (Diekman MA, 1992). Scudamore et al. (1998) analyzed various corn by-products for the presence of mycotoxins in which corn screenings yielded the highest total mycotoxin load (45,000 µg/kg) amongst ingredients. Furthermore, corn screenings are often expected to contain the highest level of myocotoxins which can lead to severe morbidity and mortality in livestock species (Richard, 2007). In swine, diseases such as pulmonary edema and hydrothorax have been associated with the higher mycotoxin concentrations in corn-screenings with higher swine mortality being observed in severe cases (Harrison et al., 1990).

While the potential presence of mycotoxins in corn screenings raises concerns for poultry producers, anti-nutritional factors such as NSP and variations in nutrient content should also be kept in mind when considering dietary inclusion. Corn screenings contain approximately 7.70% CP, 2.90% fat, 12.40% neutral detergent fiber (NDF) and 3.70% acid detergent fiber (ADF) compared to whole corn which contains 7.70% CP, 3.70% fat, 9.60% NDF, and 2.80% ADF (ADM, 2016). Although the NSP content of corn screenings has yet to be published, it can be inferred that the higher NSP levels of screenings can be associated with an increase in NDF composition compared to that of corn. While research regarding the incorporation of corn screenings in poultry diets is rather limited, the use of other grain screenings have been reported (Audren et al., 2002; Mazhari et al., 2011; Saki and Alipana, 2005; Stapleton et al., 1980). Audren et al. (2002) detected no differences in broiler performance (growth, feed efficiency, and

mortality) through 36 days of age when completely replacing wheat with wheat screenings in a balanced diet. However, due to variations in the composition of the wheat screenings used in the study, caution was recommended when incorporating screenings in broiler diets. Similarly, Stapleton et al. (1980) indicated that feeding 62% wheat screenings had no impact on BW or FCR despite the fiber content of wheat screenings ranging from 2.56 to 4.18%. In a study conducted by Saki and Alipana (2005) in which increasing levels of wheat screenings (0, 10, 20, and 30%) were fed to 21 day old Ross 308 broilers until slaughter (d 46), no differences in FC, BW, or FCR were reported. In contrast, Mazhari et al. (2011) reported no adverse effects on broiler performance during the starter phase when feeding wheat screenings at levels up to 18%; however, a reduction in BW gain was detected when incorporating higher levels of wheat screenings (24%) in broiler starter diets (d 1 to 10).

The inclusion of by-product ingredients such as DDGS and corn-screenings may become a feasible alternative for producers looking to reduce production costs. However, production and processing methods used to generate these by-products can significantly affect nutrient composition and quality with concerns over nutrient variations within ingredient batches and anti-nutritional factors such as NSP.

Anti-Nutritional Factors

Cereal grains and legumes are the predominant constituents in poultry diets with corn and soybean meal being the primary feed ingredients used in U.S. poultry production (Stefanello et al., 2016). Although these feedstuffs contribute to the majority of the diet's energy and protein requirements, the presence of varying levels of anti-

nutritive factors can impede nutrient digestion and absorption and may hinder bird performance.

Non-Starch Polysaccharides (NSP). Non-starch polysaccharides (NSP) are α-linked polymers of high molecular weight composed of both soluble and insoluble pentose and hexose sugars present in plant cell walls (Kumar et al., 2012; Sethy et al., 2015). These NSP are largely indigestible to monogastric animals such as swine and poultry and are considered anti-nutrients as they can hinder nutrient digestion and absorption resulting in sub-optimal bird performance (Choct, 1997; Williams et al., 1997). Choct (1997) reported that NSP in cereal grains are composed of predominantly arabinoxylans, B-glucans and cellulose with a higher portion of cell wall components and NSP fractions being present in cereal grain by-products such as DDGS. Similarly, Knudsen (2014) noted that grains such as wheat and corn were rich in arabinoxylans, whereas viscous grains such as barley and oats contained high levels of β-glucans. Although corn has a relatively low NSP content compared to other grains such as wheat (8.1 vs. 11.4) (Choct, 1997), its contribution to the total NSP level of the diet can be significant due to its high inclusion rate in corn-soybean meal diets (Yegani and Korver, 2013). Furthermore, the incorporation of by-product ingredients such as DDGS could further elevate total NSP concentrations with a particular increase in soluble pentosans (Loar et al., 2010). Świątkiewicz and Koreleski (2007) reported that corn DDGS contain more than 250 g/kg of total NSP with xylan being the predominant hemicellulose. The classification of NSP can be determined by their physiochemical properties such as viscosity, water-holding capacity, and molecule binding capabilities (Kumar et al.,

2012). While a complete understanding of all the anti-nutritive properties of NSP have yet to be completely identified, two models involving intestinal viscosity and nutrient encapsulation have been proposed (Williams et al., 1997).

Intestinal viscosity. When dissolved in water, soluble polysaccharides form viscous solutions by interacting directly with water molecules (Sethy et al., 2015). Viscosity within the gastrointestinal tract is determined by several factors including molecular size and conformation, NSP concentration, and surrounding structures and charged groups that are present (Smits and Annison, 1996). It has been suggested that the majority of the anti-nutritive properties of NSP, which negatively affect broiler performance, can be directly attributed to the viscosity of the aqueous fractions as a result of the viscous properties associated with soluble NSP (Choct and Annison, 1992; Williams et al., 1997). According to Bedford and Classen (1992), there is a positive correlation between increases in intestinal viscosity and growth suppression in broilers. As viscosity of the digesta escalates, the diffusion rate of digestive enzymes is reduced leading to lower overall substrate digestibility (Edwards et al., 1988). Additionally, increasing viscosity within the intestine can modify epithelial surfaces (Yaghobfar and Kalantar, 2017), increase enterocyte turnover (Smithard and Silva, 1995; Smits and Annison, 1996), reduce the passage rate of digesta (Bedford, 1996; Van Der Klis et al., 1993), and stimulate bacterial proliferation within the small intestine (Langhout, 1998; Ştef et al., 2009). As feed passaged is slowed, nutrient absorption is hindered and intestinal microbiota are allowed to proliferate and migrate to a more anterior location within the small intestine where they compete with host for essential nutrients such as

starch and protein (Bedford, 1995). In a recent review, Kheravii et al. (2018) suggested that concentrations of water soluble NSP serve as substrates for intestinal bacteria, particularly pathogenic species such as *C. perfringens* which can predispose birds to disease. Langhout et al. (2000) conducted a study in which birds were fed an NSP source (highly methylated citrus pectin (HMC)) at 30 g/kg in a corn-soybean meal diet through 21 days of age. The authors noted that feeding HMC increased digesta viscosity within the small intestine reduced amino acid and energy digestibility, increased proliferation of intestinal microflora, and depressed weight gain and feed utilization (MJ/kg gain) by 9.5 and 15.2%, respectively. In a similar experiment directed by Choct and Annison (1992), the inclusion of 35 g of alkali-extractable pentosans (854 g arabinoxylans/ kg DM) per kg of diet elevated digesta viscosity which hindered starch, lipid, and nitrogen digestibility, and negatively impacted broiler weight gain and FCR.

Nutrient Encapsulation. The physical entrapment of essential nutrients is believed to be a mechanism of action regarding NSP, specifically insoluble NSP. Unlike soluble NSP, insoluble NSP fractions are not water-soluble and therefore are not likely associated with viscosity (Meng et al., 2005; Sethy et al., 2015). However, the presence of these insoluble cell wall fractions can result in the physical encapsulation of highly digestible nutrients such as starch and amino acids rendering them inaccessible to the bird (Ohimain and Ofongo, 2014; Theander et al., 1989). McAllister and Cheng (1996) reported that within the endosperm, starch granules are embedded in a protein matrix and surrounded by cell walls limiting their digestion and absorption. In corn, insoluble NSP (51 g/kg insoluble arabinoxylan) are the primary cell wall components with soluble

NSP content being rather minimal (1 g/kg arabinoxylan) (Choct, 1997). Bedford (2002) stated that the degree of nutrient encapsulation is dependent upon the concentration of insoluble arabinoxylans located in cereal endosperm cell walls. Additionally, Simon (1998) concluded that pre-cecal starch digestibility appeared to be the most ideal indicator of successful cell wall degradation when evaluating enzyme efficacy since starch is the primary nutrient located in the endosperm of cereal grains. Svihus (2014) determined that the low digestibility of starch in wheat-based diets was partly associated with the entrapment of starch granules in the protein matrix and cell walls. In a recent experiment, Stefanello et al. (2015) indicated a 3.5% and 2.4% improvement in starch digestibility in the jejunum and ileum respectively, when feeding an amylase + xylanase combination in corn-soy diets which was attributed to increased cell wall fragmentation. The physical entrapment of nutrients by insoluble NSP can result in undigested starch, referred to as "resistant starch" (Englyst et al., 1982), as well as inaccessible protein substrates leading to lower nutrient availability and poor bird performance.

Phytate. In cereal grains and legumes, approximately 60-80% of the total P is stored in the form of phytate (*myo-inositol 1, 2, 3, 4, 5, 6-hexakisdihydrogen phosphate; IP6*) with total phytate-P concentrations ranging from 8.0 to 9.0 g/kg in standard corn-soy based poultry diets (Cabahug et al., 1999; Cowieson et al., 2004). Furthermore, phytate levels within the diet have the potential to increase greatly when incorporating alternative ingredients such as cereal grain by-products (Cabahug et al., 1999). The structure of phytate consists of twelve reactive sites that possess strong negative charges which are able to bind with di- and trivalent minerals (calcium, zinc, manganese, and

iron) as well as proteins and starch, therefore, reducing the availability of both the nutrient and phytate to animals (Angel et al., 2002; Pallauf and Rimbach, 1997). In an overview written by Maenz (2000), it was proposed that an inverse relationship existed between dietary phytate and mineral digestibility as chelation among phytate and multivalent cations formed insoluble complexes rendering them unavailable to the animal. Cowieson et al. (2006a) noted an increase of 187, 39, 87, and 174% in the excretion of Ca, Mg, Mn, and Na, respectively when feeding 1 g of phytate (IP6) to birds. In poultry, absorption and digestion of P from phytate is minimal as birds do not possess adequate endogenous enzymes capable of phytate hydrolysis (Bedford, 2000; Cowieson et al., 2006a; Ravindran et al., 1995). This insufficient uptake of P results in approximately 70% of the total P in feed being released in the excreta (Turner et al., 2002). Furthermore, the anti-nutritive properties associated with phytate can negatively impact the digestion of minerals, energy, and amino acids through chelation and the formation of complexes (Selle et al., 2000), while also increasing the excretion of endogenous compounds.

The formation of protein-phytate complexes is primarily pH dependent with binary protein-phytate complexes being formed at low pH and ternary protein-phytate complexes forming as pH increases (Reddy and Salunkhe, 1981; Selle et al., 2000). Cowieson et al. (2006a) noted a 12% reduction in true amino acid digestibility when feeding birds varying combinations of casein and phytate. It was suggested that these results might be attributed to several mechanisms including phytate-protein interactions, and the inhibition of endogenous enzymes and their cofactors, resulting in inadequate

digestion of dietary proteins. In a meta-analysis of twenty-four peer-reviewed papers, Cowieson et al. (2017), detected a negative correlation between phytate-P concentration and amino acid digestibility when phytate-P levels ranged from 0.14 to 1.56%. Furthermore, it has been suggested that the endogenous protein loss associated with phytate may negatively impact digestible energy by as much as 0.1 MJ/kg of DM intake for every 1 g/kg of dietary phytate P (Cowieson and Ravindran, 2007). The presence of phytate within the gastrointestinal tract may also increase the secretion of endogenous enzymes and gastrointestinal mucin, therefore increasing the excretion of endogenous amino acids and minerals (Cowieson et al., 2004). In an experiment conducted by Onyango et al. (2008), birds fed two forms of phytic acid exhibited an elevated endogenous loss of mucin, sialic acid, and various amino acids. Similarly, Cowieson et al. (2004) reported an increase in mucin production and higher excretions of amino acids and minerals (Na, Fe, S, and Ca) when feeding broilers sodium phytate. The authors suggested that the presence of phytate within the gut might have encouraged epithelial irritation leading to greater endogenous losses of amino acids due to higher secretions of mucin, which is relatively rich in various amino acids such as serine, threonine, proline, and cysteine. Although the mode of action is not fully understood, it is believed that phytate may also negatively influence energy utilization (Selle et al., 2006). Wu et al. (2015) hypothesized that the endogenous losses from phytate may increase the energy required for maintenance, thus, reducing energy availability for growth. Furthermore, phytate can bind to energy-generating constituents such as carbohydrates and lipids while indirectly impacting energy digestibility through the binding of endogenous

enzymes and cofactors involved in energy production (Selle and Ravindran, 2007; Thompson et al., 1987). An additional mechanism was proposed by Ravindran et al. (2000) stating that phytate can potentially form mineral-phytate complexes which can increase soap formation and hinder energy utilization through reductions in lipid digestibility. In a study evaluating low (0.22%) and high (0.48%) phytate levels in pigs, Liao et al. (2005) observed a 4.7% reduction in ileal digestible energy when feeding growing pigs diets high in phytate. Additionally, Ravindran et al. (2006) noted reductions in AME with increasing phytate concentrations in broiler diets.

It has been recognized that the anti-nutritive properties of phytate can reduce nutrient absorption and negatively impact broiler performance. Cabahug et al. (1999) evaluated three concentrations of phytic acid (10.4, 13.2, and 15.7 g/kg) when fed to male broilers from day 7 to 25 in which increasing levels of phytic acid depressed both FC and BW gain. Additionally, Liu et al. (2008) published results describing a 73 g/bird reduction in BW and an adverse effect on FCR (1.40 vs. 1.50) when increasing phytate-P levels from 0.22% to 0.44% in 28 day old broilers. dos Santos et al. (2014) noted improvements in BW gain (607 vs. 480 g), FCR (1.445 vs. 1.460) and higher FC (877 vs. 701g) in birds fed low phytate (6.40 g/kg) compared to high phytate (10.65 g/kg) levels in low avP (1.80 g/kg) diets from 0 to 21 d of age.

Use of Exogenous Enzymes

Since the mid 1980's, the commercial use of exogenous enzymes in poultry diets has been practiced in an effort to increase the feeding value of raw ingredients, reduce variation in nutrient quality of feedstuffs, and improve overall diet costs (Bao et al.,

2013; Bedford, 2000). Over the past decade, the feed enzyme market has expanded substantially in response to rising ingredient costs (Ravindran, 2013).

NSP enzymes. The global use of non-starch polysaccharide degrading enzymes (NSPase) has become widely accepted with over 70% of feed manufacturers incorporating NSPase into poultry diets. Furthermore, there are a variety of NSPase products commercially available to the feed industry ranging from single component enzymes (e.g. xylanase and β-glucanase) that possess only one enzyme activity, to "cocktail" NSPase enzymes involving a product of a single fermentation which possesses several enzymatic activities (Aftab and Bedford, 2018; O'Neill et al., 2014). There are numerous publications reporting the beneficial impact of NSPase inclusion on nutrient digestibility, intestinal health, broiler performance, and diet costs (Bedford and Classen, 1992; Campasino et al., 2015; Cozannet et al., 2018; De Keyser et al., 2016; O'Neill et al., 2012b; Toghyani et al., 2017; Zhang et al., 2012). O'Neill et al. (2012b) stated that the incorporation of NSPase in poultry diets could lower diet costs by removing energy through fat substitution or dilution of the diet using high-fiber feed ingredients. Additionally, Ravindran (2013) suggested NSPase inclusion could allow for increased incorporation of lower-quality feed ingredients without sacrificing broiler performance. These positive responses have been suggested to derive from two principle modes of action regarding NSPase addition including the reduction in intestinal viscosity and the degradation of plant cell walls (O'Neill et al., 2014). NSPase enzymes hydrolyze NSP fractions that are usually indigestible to monogastric species, improving nutrient

availability and mitigating the anti-nutritive properties associated with NSP (Simon, 1998).

Xylanase. Xylanase is a single component, NSP enzyme that has been supplemented in poultry diets in order to degrade complex polysaccharides located in the cell wall of plant-based feed ingredients. Arabinoxylans are the predominant NSP found in the cell walls of cereal grains and are comprised of a xylan backbone with side chains consisting of arabinose, uronic acid and other sugar residues (Morgan et al., 2018; Shibuya et al., 1983). In corn-soy diets, total arabinoxylan content has been estimated to be approximately 5.2% in corn (Choct, 1997) and 3.3% in soybean meal (Knudsen, 1997). The addition of xylanase initiates the breakdown of arabinoxylans by depolymerizing the xylan backbone via hydrolysis of the β-1, 4 glycosidic bonds between the xylose residues and the main chain (Berrin and Juge, 2008). Successful degradation of arabinoxylans allows for greater nutrient access, reductions in digesta viscosity, and improvements in broiler performance (Choct and Annison, 1992; Choct et al., 2004; Lei et al., 2016; Zhang et al., 2014). Although xylanase supplementation has been well established in wheat-based diets (Bedford and Schulze, 1998), improvements in broiler performance have been noted in corn-based diets as well. Several works have been published highlighting the beneficial impact of xylanase on broiler performance when included in low-energy corn-soy diets (Coppedge et al., 2012; Flores et al., 2017; Williams et al., 2014b). Liu et al. (2011) detected an increase in BW and enhanced digestibility of hemicellulose and energy by 20% and 620 kJ/kg, respectively, when including xylanase in corn-soy diets with increasing levels of corn DDGS (0, 100, and

200 g/kg). Additionally, Amerah et al. (2017) described a 49 g increase in BW gain and a reduction in cumulative FCR (d 1 to 42) when including xylanase at 2,000 units per kilogram (U/kg) in energy/amino acid deficient diets. While it is unlikely that these improvements in performance are associated with a reduction in intestinal viscosity due to the low soluble NSP content in corn-soy diets (Choct, 1997), improvements may be attributed to cell wall degradation. Cowieson (2005) suggested that the favorable responses observed with xylanase addition in corn-soy diets are likely contributed to the release of encapsulated nutrients through cell wall hydrolysis rather than reductions in gut viscosity. Although corn-soy diets are considered to be highly digestible, there is still approximately 400 to 450 kcal/kg of undigested energy that is potentially available for utilization via exogenous enzyme supplementation (Cowieson, 2010). Stefanello et al. (2016) observed an increase of 145 kcal/kg in IDE, as well as an improvement of 5% and 2.6% in crude protein and fat digestibility, respectively, when adding 100 FXU/kg of xylanase in corn-soy diets. Similarly, O'Neill et al. (2012a) discovered a 17 g increase in BW gain and a 2 point reduction in FCR as a result of an improvement of 133 kcal/kg in IDE when supplementing 16,000 units per kilogram (U/kg) of xylanase in corn-soybean meal diets. These results indicate that xylanase supplementation in corn-soy diets can improve nutrient utilization through cell wall degradation resulting in improvements in broiler performance.

Cocktail NSPase. The use of enzyme cocktail preparations has been suggested to represent the next generation of feed enzymes as a strategy to improve nutrient utilization in poultry diets (Ravindran, 2013). Although criticized, one common

approach when supplementing NSPase is the selection of enzyme preparations based on the variety of NSP substrates or "complexity" of the diet (Aftab and Bedford, 2018). Wu et al. (2017) speculated that due to a wider variety of plant-based ingredients used in modern poultry diets, the administration of enzyme combinations with additional activities, each differing in substrate affinity, may further enhance the utilization of nutrients compared to xylanase alone. Furthermore, the incorporation of higher levels of by-product ingredients that are lower and/or inconsistent in nutritional quality can increase the amount and variation of substrate in the diet, therefore increasing the overall enzyme response (Choct, 1997; Jimoh, 2018). In a study conducted by Williams et al. (2014a), the authors discovered that intermittent application of a β- mannanase (d 1 to 21) and a cocktail NSPase (d 22 to 47) yielded further improvements in broiler performance compared to individual inclusion when targeting specific substrates determined by a dietary ingredient profile in corn-soy diets. Similarly, Meng et al. (2005) reported the greatest improvement in FCR and AME when supplementing a multi-carbohydrase cocktail enzyme containing 1,000 units of xylanase, 400 units of glucanase, 1,000 units of pectinase, and small amounts of cellulase, mannanase, and galactanase compared to individual combinations when fed to broiler chicks from 5 to 18 days of age.

The use of NSPase allows for the removal of energy through fat substitution or nutrient dilution of the diet (O'Neill et al., 2012b). Coppedge et al. (2012) reported increases in broiler body weight when supplementing a cocktail NSPase (1,500 units of xylanase, 1,100 units of β-glucanase, 35 units of α-galactosidase, and 110 units of β-

mannanase) in reduced energy (-133 kcal/kg) diets fed to male broilers. Zou et al. (2013) fed birds a combination of xylanase (3,000 U/kg) and β-glucanase (400 U/kg) in low energy (-100 kcal/kg) diets in which the enzyme combination improved AME resulting in FCR similar to that of the standard energy diet. Similarly, Yu and Chung (2004) evaluated a cocktail NSPase consisting of α-amylase, xylanase, and β-glucanase when included in low-energy (-93 kcal/kg) corn-soybean meal diets fed to broilers through 38 days. When supplementing cocktail NSPase, the authors noted a 53 g increase in BW gain and an 8 point reduction in FCR confirming a reduction in dietary energy is possible without sacrificing broiler performance. As feed costs continue to increase, the addition of NSPase in diets with greater levels of by-product ingredients is becoming more common. Romero et al. (2014) acknowledged that increasing ingredient prices have intensified the demand for substitute ingredients such as DDGS resulting in more nutritionally "complex" and variable diets compared to conventional corn-soy diets. Campasino et al. (2015) evaluated a cocktail NSPase (xylanase, β-glucanase, and α-galactosidase) in low-energy (-132 kcal/kg) diets containing levels of DDGS ranging from 2.5 to 10 percent when fed to broilers through 48 days of age. The addition of cocktail NSPase improved broiler performance (BW and FCR) as well as processing parameters (carcass weight, breast and tender weight) to levels similar to that of the standard control diet. Similarly, Klein et al. (2015) noted a 40 g increase in BW and a 1.5 point reduction in FCR (d 1 to 35) at d 35 when including a combination of cocktail NSPase and β-mannanase in reduced energy diets containing 2.5% DDGS. These

improvements in performance resulted in a 74 and 60 g increase in live and carcass weight, respectively.

Phytase. Phytase has been supplemented in poultry diets in order to hydrolyze the phytate bonds allowing the bird access to the plant phytate phosphorus (P), in turn, reducing the anti-nutritive impact of phytate, improving P digestibility and decreasing the inclusion of inorganic P sources (Bedford, 2000; Selle et al., 2000; Slominski, 2011). Depending on source (fungal or microbial), phytase catalyzes the hydrolysis of phytate (myoinositol-1, 2, 3, 4, 5, 6-hexakisphosphate) at either the 3- or 6-position of the inositol ring initiating the sequential release of the remaining five phosphate groups yielding lower phytate esters and ultimately, in theory, inositol and inorganic P (Selle et al., 2000; Shanmugam, 2018). It is suggested that phytate hydrolysis occurs primarily in the fore-stomach (crop, proventriculus, and gizzard) with hydrolysis of phytate by exogenous phytase occurring mainly in the crop (Onyango et al., 2005; Selle and Ravindran, 2007). Manangi and Coon (2008) reported that the inclusion of a microbial phytase at 500 FTU/kg resulted in 74.7% hydrolysis of phytate. Beeson et al. (2017) recorded improvements in phytate degradation with the addition of 500 FTU/kg reducing IP6, IP5, and IP4 phytate esters. Although dependent on dietary phytate levels (Ravindran et al., 1995), phytase source and concentration (Shirley and Edwards, 2003; Simons et al., 1990), general improvements in P availability from phytate hydrolysis range between 20 to 40% with phytase supplementation (Singh, 2008). The degradation of phytate allows for the release of inorganic P as well as chelated minerals, amino acids and starch bound to the phytate molecule (Selle et al., 2000; Viveros et al., 2002). Santos

et al. (2008) observed enhanced digestibility of calcium (Ca) and P (22.7 and 36.9% increase, respectively) when supplementing 500 U/kg of phytase in low nutrient (2,995 kcal/kg, 0.84% Ca, 0.55% tP) diets. Akter et al. (2018) reported an increase of 3 and 8% in ileal digestibility of Ca and P, respectively, when including 500 FTU/kg of phytase in diets with varying levels of dietary Ca (6, 8, or 10 g/kg) and non-phytate P (3 or 4 g/kg). Additionally, Lalpanmawia et al. (2014) observed a 17.2 and 5.3% improvement in apparent ileal digestibility of P and Ca, respectively, when incorporating a commercial fungal phytase at 500 FTU/kg in low P (3.2 g/kg avP) corn-soy diets.

Although mineral utilization was originally the focus with phytase supplementation, increasing research has indicated improvements in energy and amino acid availability as well. Kornegay et al. (1999) reported a linear increase in the digestibility of amino acids when supplementing increasing levels of phytase (150, 300, and 450 FTU/kg) in amino-acid deficient diets. Similarly, Ravindran et al. (2006) observed improvements in ileal protein and amino acid digestibility with phytase addition regardless of phytate concentrations (10.4, 11.8, and 13.6 g/kg). Although not fully understood, it is believed that phytase may inhibit the formation of protein-phytate complexes (Selle et al., 2000) and limit the secretion of endogenous amino acids (Cowieson et al., 2004) therefore, improving amino acid retention and digestibility. Ravindran et al. (1999) reported that the improvements in amino acid digestibility with phytase addition could be attributed to reduced endogenous amino acid secretion as a result of the removal of phytate and its antinutritive properties. Furthermore, Amerah et al. (2014) verified a positive correlation ($P < 0.05$) between the digestibility of amino

acids and the degree of phytate degradation with phytase supplementation. Rutherfurd et al. (2004) assessed the effect of a microbial phytase (500 and 750 U/kg) in low P diets (0.35 avP) in which phytase addition increased phytate hydrolysis (11% greater disappearance) and improved the ileal digestibility of all amino acids except methionine, tyrosine, histidine, and tryptophan. Similarly, Camden et al. (2001) detected a linear increase in apparent ileal digestibility of all amino acids (except cystine, glutamic acid, glycine, leucine, proline, and serine) and energy when feeding graded levels of phytase (250, 500, and 1,000 U/kg) in low P (0.30% avP) and Ca (0.80%) diets. Reports identifying improvements in energy utilization with phytase addition have been published as well (Amerah et al., 2014; Namkung and Leeson, 1999). Ravindran et al. (2000) observed an increase in AME values by 1.3% in low, non-phytate P (2.3 g/kg) and by 5.7% in adequate, non-phytate P (4.5 g/kg) diets when supplementing a microbial phytase. In a subsequent trial, Ravindran et al. (2001) and colleagues noted that the AME content of the diet was improved with increasing levels of phytase to 750 FTU/kg when fed to broilers. Similarly, Selle et al. (2007) detected a 2.4% increase in AME (14.56 vs. 14.22 MJ/kg) when incorporating 500 FTU/kg of phytase in adequate P diets containing 10.0 and 11.8 g/kg lysine fed to broilers from day 7 to 28 of age.

Bone development has been considered an ideal test for estimating the bioavailability of minerals such as Ca and P (Ammerman, 1995). More specifically, tibia ash concentration is often used to determine the degree of bone mineralization in broilers and has been suggested as a more sensitive indicator compared to other quantification methods (Shastak et al., 2012). For example, reducing the P content by 1 g/kg (3.5 to 2.5

g/kg) in the diet can decrease tibia ash by 1% (Brenes et al., 2003). Numerous studies have shown improvements in tibia ash content with the addition of phytase in corn-soy diets (Powell et al., 2011; Santos et al., 2008; Sebastian et al., 1996; Sousa et al., 2015; Viveros et al., 2002; Walk et al., 2011). Tang et al. (2012) conducted an experiment evaluating a novel thermostable phytase in P deficient corn-soy diets in which 500 FTU/kg increased tibia ash and tibia P content. Brenes et al. (2003) published data indicating phytase increased tibia ash (up to 4%), Ca (up to 2%), P (up to 1%) and zinc (up to 4%) content when evaluating three levels of phytase (200, 400, and 600 U/kg) in corn-soy diets containing two levels of avP (3.5 and 2.5 g/kg) and two levels of citric acid (0 and 20 g/kg).

It is widely accepted that the addition of phytase increases growth performance in broilers with numerous publications reporting improvements (dos Santos et al., 2014; Powell et al., 2011; Tang et al., 2012; Vieira et al., 2015; Wu et al., 2004a). Selle et al. (2007) noted a 16 g increase in FC (g/bird) yielding a 29 g improvement in BW gain and a 4 point reduction in FCR when supplementing 500 FTU/kg of phytase in corn/wheat diets. Similarly, Lu et al. (2009) detected a 3.1 g increase in FC (d 1 to 35) resulting in a 7.5% increase in BW gain (44.7 vs. 41.6 g/d) and a 7 point reduction in FCR when feeding 500 FTU/kg in low P (0.15% avP reduction) corn/wheat diets. Although phytase has been known to improve bird performance, several factors including Ca:avP ratio and dietary Ca and P content have been suggested to contribute to the variations in the degree of response (Bedford et al., 2016; Delezie et al., 2015; Driver et al., 2005). Driver et al. (2005) determined that the concentrations of Ca and P in the diet are the most

important factors in determining the response to phytase addition. A literature review by Selle and Ravindran (2007) suggested that phytase addition in P deficient diets may yield a more pronounced improvement in performance compared to diets with higher P levels. Furthermore, the authors concluded that FC and weight gain responses with phytase addition were more robust and less variable than feed efficiency responses. While FCR improvements are less common, improvements in BW gain have been suggested to correspond with FC as dietary P deficiency is mitigated (Waldroup et al., 2000; Walk et al., 2014). Rosen (2002.) conducted an extensive review determining that phytase inclusion in diets resulted in an average increase of 81 g in FC and a 57.7 g improvement in BW gain. In an experiment conducted by Panda et al. (2007), the inclusion of 500 FTU/kg of phytase elicited a 146 g increase in FC (g/bird) resulting in a 127 g improvement in BW gain when feeding low P (0.30% avP) in corn-soy diets. Additionally, the authors determined that the addition of phytase yielded a greater response as P levels were reduced (0.35 to 0.30% avP). Woyengo et al. (2010) evaluated the addition of 600 FTU/kg of phytase in low P (0.26% avP) diets with the inclusion of enzyme numerically increasing FC (g/bird) by 41.1 g generating a 38.1 g improvement in BW gain when fed to broilers through 21 days.

Super-dosing phytase. Although the use of conventional doses of phytase (500 FTU/kg) has been supplemented in poultry diets over the past several decades, the practice of super-dosing (> 1,500 FTU/kg) has gained increasing attention by poultry producers with the decrease in phytase cost. Previous studies have reported further improvements in nutrient utilization and broiler performance when super-dosing phytase

compared to conventional doses (Augspurger and Baker, 2004; Shirley and Edwards, 2003; Walk et al., 2013). These improvements observed with super-dosing phytase have been suggested to be attributed to primarily three mechanisms involving: 1.) increased liberation of bound inorganic phosphate or restoration of P/Ca proportionate release, 2.) less residual phytate and its antinutritive properties, and 3.) the potential generation of myo-inositol with vitamin-like/lipotropic effects (Cowieson et al., 2011; Shirley and Edwards, 2003). Data from Shirley and Edwards (2003) evaluating increasing doses of phytase (up to 12,000 U/kg) suggest that higher levels of phytase can improve phytate disappearance, nitrogen and mineral retention, as well as improved energy utilization. Additionally, Walk et al. (2014) noted almost complete hydrolysis of phytate (IP6) and an increase in inositol concentration resulting in improved body weight gain and FCR when supplementing higher doses of phytase (1,000 and 1,500 FTU/kg). In a study directed by Manangi and Coon (2008), the authors observed a 92.77, 96.91, and 99.45% hydrolysis of phytate when supplementing 1,500, 2,000, and 5,000 FTU/kg, respectively, compared to a 74.79% hydrolysis of phytate in diets with 500 FTU/kg of phytase inclusion. This increase in phytate degradation associated with higher levels of phytase resulted in a further increase in FC and heavier BW compared to birds fed 500 FTU/kg. It has been well accepted that beyond meeting the P requirement, phytase supplementation can elicit further beneficial responses known as “extra-phosphoric effects” including improvements in amino acid and energy digestibility, mineral retention, and performance responses (Cabahug et al., 1999; Gehring et al., 2013). Cowieson et al. (2011) suggested that phytase increases FC in P deficient diets in order

to meet the birds requirement, however, the inclusion of super-doses of phytase continues to stimulate intake beyond its P needs. In a study conducted by Pieniazek et al. (2017), the authors evaluated the inclusion of phytase at 500 and 2,000 U/kg in low-P diets on nutrient digestibility and performance. Data from the trial demonstrated that super-doses of phytase (2000 U/kg) improved amino acid digestibility to levels similar to that of the control diet while yielding a 9.1% increase in FC (4.352 vs. 3.988 kg/bird) resulting in a 142 g heavier d 42 BW compared to conventional doses (500 U/kg). Similarly, Manobhavan et al. (2015) reported higher mineral deposition and a 10.4 and 14.9% increase in FC resulting in a 163 and 196 g heavier BW gain, respectively, with phytase supplementation at 2,500 and 5,000 FTU/kg compared to levels of phytase at 500 FTU/kg. Based on results obtained from previous literature (Cowieson et al., 2006b; Gehring et al., 2013; Pieniazek et al., 2017; Pirgozliev et al., 2011; Pirgozliev et al., 2010; Pirgozliev et al., 2008), it can be speculated that super-dosing phytase may further enhance nutrient digestibility and animal performance beyond that of conventional levels fed in broiler diets.

The use of alternative ingredients in broiler diets has become a necessary step for producers needing to reduce feed costs during times of high feed ingredient costs. Although exogenous enzyme supplementation has been practiced for the last several decades, the incorporation of lower quality feed ingredients as well as the need to reduce the nutrient density of the diet has warranted further research to identify optimum enzyme inclusion strategies. The purpose of the research described herein was to evaluate the inclusion of NSPase in diets fed to broiler chickens with low quality, by-

product ingredients, as well as determine the impact of increasing doses of phytase in low P diets.

CHAPTER III

EVALUATION OF NSPASE INCLUSION IN DIETS MANUFACTURED WITH HIGH AND LOW QUALITY CORN ON MALE BROILERS*

Introduction

In the ever continuous effort to reduce diet costs, alternative ingredients are commonly used in U.S. broiler diets. Corn screenings are a corn by-product obtained from repetitious screenings of corn following cleaning and manufacturing. Prior to exportation, whole corn is harvested and dried to lower moisture content to 14 to 15 percent to significantly reduce mold, bacteria, and sprout deterioration during storage (Lin and Lin, 1994). Following the drying process, corn is cleaned and sieved to remove any broken grain, grain dust, chaff, and various weed seeds resulting in a screening by-product (Lin and Lin, 1994). These screenings have become an ideal alternative feed ingredient for poultry producers as they are approximately 70 to 80 percent of the cost of whole corn. This cost reduction is mainly due to the variation in nutrient content between different loads of screenings which contain a higher percentage of fiber due to the inclusion of "bee wings" and chaff, lower caloric values, and higher moisture content resulting in an overall lower nutrient value when compared to whole kernel corn (Hess et al., 1999; Lin and Lin, 1994). It is hypothesized that the higher fiber content found in

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corn screenings would suggest that the concentration of nonstarch polysaccharides (NSP) would be higher as well.

Nonstarch polysaccharides are major components of dietary fiber found in feed ingredients that consist of both soluble and non-soluble polysaccharides. In corn, levels of soluble and insoluble NSP are 0.1 and 8.0% respectively with the majority being arabinoxylans and ß-glucans (Choct, 1997; Slominski, 2011). While corn is not considered a viscous grain, its contribution to the overall NSP content of the diet can be substantial due to its high inclusion rate (Yegani and Korver, 2013). Nonstarch polysaccharides possess anti-nutritive properties that can hinder bird performance and alter gut physiology. These anti-nutritive properties include high gut viscosity, encapsulation of starch and amino acids, sticky droppings, modification of gut physiology, and an overall reduction in bird performance (Choct, 1997; Meng et al., 2005; Slominski, 2011). It is presumed that the inclusion of a higher fiber ingredient such as corn screenings could increase the overall NSP content of the diet which could further exaggerate the anti-nutritive properties associated with NSP. In order to ameliorate the anti-nutritive effects of NSP and improve bird performance, nonstarch polysaccharide degrading enzymes (NSPase) have been supplemented in poultry diets (Avila et al., 2012; O'Neill et al., 2012b; Williams et al., 2014a).

The commercial use of exogenous enzymes within the poultry industry have been practiced since the 1980's to enhance digestibility, absorption, and utilization of various nutrients within feed ingredients (Choct, 2006). It is believed that the supplementation of exogenous enzymes in the diet can yield numerous improvements including increasing

the range of feedstuffs that can be used within a diet, reducing variability in nutritive value between batches of ingredients, and improving the digestion and absorption of nutrients (Ravindran, 2013). Nonstarch polysaccharide degrading enzymes cleave various sites within the polysaccharide chain which reduce viscosity and enable access to entrapped starch and amino acids (Meng et al., 2005). Although it is generally accepted that corn-soy diets are highly digestible, improvements in nutritional value can still be achieved through NSPase supplementation (Café et al., 2002; Gracia et al., 2003; Zanella et al., 1999). Previous research has shown that supplementing NSPase in corn-soy diets can alleviate the anti-nutritive properties associated with NSP and improve bird performance (Coppedge et al., 2012; Leslie et al., 2007; Olukosi et al., 2010). One factor that must be taken into consideration when supplementing NSPase is the substrate variability between ingredients. Substrate type and content differ between ingredients and may respond differently to enzyme application resulting in different interactions and results. It is believed that enzyme response is dependent on the quality of the ingredient being included in the diet. Lower quality ingredients such as corn screenings allow a greater enzyme response and therefore yield greater improvements when compared to higher quality feed ingredients (Ravindran, 2013).

Materials and Methods

Experimental Design

On day of hatch, 1,950 Cobb 500 males were allotted to floor pens and dietary treatments based on initial body weight. Each treatment consisted of 16 replicates containing 20 birds per replicate pen. Chicks were provided supplemental heat and given access to feed and water *ad libitum*. Chicks were placed in 0.91m x 1.83m rearing pens with tube feeders and nipple drinkers with fresh pine shavings as bedding material. Animal care was provided in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC). The dietary program consisted of 3 dietary phases with a starter diet being fed from 1 to 13 d of age, grower from 14 to 27 d, and a finisher being fed from d 28 to 42. All broilers and feed were weighed on the days of dietary changes for calculation of average body weight (BW) and determination of feed consumption (FC) for the calculation of mortality corrected feed conversion ratio (FCR).

Experimental Diets

The effect of NSPase[1] inclusion in diets manufactured with high and low quality corn was evaluated in a completely randomized experimental design consisting of 6 dietary treatments through a 42 d grow-out period. Low quality corn was achieved with the inclusion of 20% corn screenings and formulations were made on an isonitrogenous and isocaloric basis (Table III-1 and Table III-2). Diets were formulated based on a 2 X 3 factorial consisting of diet type (presence/absence of corn screenings) and energy

[1] Enspira®- United Animal Health, Sheridan, IN

level. Treatments included a high energy (HE), low energy (LE) with a 110 kcal/kg metabolizable energy reduction compared to the HE, and the LE supplemented with NSPase (113.5 g/ton; 2700 U/g of xylanase from *Aspergillus niger* and *Trichoderma reseei*. Also contains ß-glucanase and α- galactosidase) (LEN). One unit of xylanase activity is defined as micromoles of total reducing sugars released per minute at 40°C and pH 4.5. Diets were corn and soybean meal based with the inclusion of corn distiller's dried grains with solubles (DDGS) and meat and bone meal throughout the trial. During feed manufacturing, four large basal diets were mixed with the LE diets being divided equally prior to enzyme inclusion. All diets contained 250 FTU/kg of phytase[2] with a matrix value of 0.12% available P and 0.07% Ca. The experimental NSPase was blended with corn starch to form a premix which was included in diets at 453.6 g/ton for all dietary phases with xylanase recovery being reported in Table III-3 and Table III-4. All diets were pelleted with exception of the starter diet which was pelleted and then crumbled. The conditioning time was 10 seconds and pelleting temperature did not exceed 70°C to preserve enzyme activity. Samples were collected in duplicate during feed manufacturing for nutrient analysis. Crude protein was determined using AOAC by combustion (AOAC 990.03), total P determined by wet ash ICP (AOAC 985.01M), acid detergent fiber determined using an ANKOM digestion unit (AOAC 973.18), and an ether extraction to determine crude fat (AOAC 920.39).

[2] OptiPhos® PF 2000- Huvepharma, Peachtree City, GA

Table III-1. Ingredient profile and calculated nutrient concentration of reduced-energy diets (starter and grower phase) containing corn screenings fed to male broilers.

	Starter				Grower			
Nutrient	**HE[1]**	**HE+ CS[3]**	**LE[2]**	**LE+ CS[3]**	**HE[1]**	**HE+ CS[3]**	**LE[2]**	**LE+CS[3]**
Corn	55.64	33.30	58.43	36.10	61.54	40.52	64.04	43.31
Dehulled Soybean Meal (48%)	30.32	31.61	29.81	31.09	25.72	25.09	25.26	24.58
DL-Methionine (99%)	0.33	0.32	0.33	0.32	0.26	0.26	0.25	0.26
Lysine HCL	0.21	0.17	0.22	0.18	0.19	0.18	0.20	0.19
Fat, Blended	3.25	4.38	0.96	2.09	2.69	3.38	0.50	1.09
Calcium Carbonate	1.00	1.00	1.00	0.96	0.90	0.62	1.00	0.63
Monocalcium Phosphate	1.95	0.44	0.43	0.43	0.35	0.12	0.34	0.113
Salt	1.86	0.42	0.41	0.42	0.35	0.32	0.19	0.31
Sodium Bicarbonate	0.00	0.00	0.00	0.00	0.10	0.12	0.32	0.13
Trace Minerals[4,5,6]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vitamins[7]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salinomycin[8]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
DDGS	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Phytase[9]	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013
Corn Screenings	0.00	20.00	0.00	20.00	0.00	20.00	0.00	20.00
Meat and Bone Meal	3.00	3.00	3.00	3.00	2.50	4.00	2.50	4.00
Calculated Nutrient Concentration								
Protein	22.87	22.87	22.87	22.87	20.80	20.80	20.80	20.80
Lysine	1.32	1.32	1.32	1.32	1.16	1.17	1.16	1.17
Methionine	0.67	0.66	0.67	0.66	0.58	0.59	0.58	0.58
TSAA	1.02	1.01	1.02	1.01	0.90	0.90	0.90	0.90
Threonine	0.88	0.88	0.88	0.88	0.78	0.78	0.78	0.78
Calcium	0.92	0.92	0.92	0.92	0.82	0.82	0.85	0.82
Available Phosphorus	0.46	0.46	0.46	0.46	0.41	0.41	0.41	0.41
Total Phosphorus	0.58	0.56	0.59	0.57	0.54	0.51	0.54	0.52
Sodium	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Metabolizable Energy (kcal/kg)	3058	3058	2948	2948	3113	3113	3003	3003

[1]High Energy (HE) - Standard corn/soybean meal diet; [2]Low Energy (LE)-Energy reduced by 110kcal/kg compared to HE

[3]Corn screenings were included at 20% in the diet

[4] Trace mineral premix added in the starter yields 180 mg of manganese, 108 mg of total zinc, 5.1 mg of copper, 3.51 mg of iodine, 0.3 mg of total selenium, 0.013 g of *bacillus subtilis*

[5] Trace mineral premix added in the grower yields 150 mg of manganese, 90 mg of total zinc, 4.25 mg of copper, 2.92 mg of iodine, 0.25 mg of total selenium, 0.011 g of *bacillus subtilis*
[6] Trace mineral premix added in the finisher yields 90 mg of manganese, 54 mg of total zinc, 2.55 mg of copper, 1.75 mg of iodine, 0.15 mg of total selenium, 0.007 g of *bacillus subtilis*
[7] Vitamin premix added at this rate yields 7,700 IU vitamin A, 5,500 ICU vitamin D_3, 55 IU vitamin E, 1.5 mg vitamin K-3, 0.01 mg B_{12}, 6.6 mg riboflavin, 38.5 mg niacin, 9.9 mg d-pantothenic acid, 0.88 mg folic acid, 2.75 mg pyroxidine, 1.54 mg thiamine, 0.08 mg biotin per kg diet
[8]Active drug ingredient salinomycin sodium, 60 g/lb. (60 g/ton inclusion; Huvepharma, St. Louis, MO). As an aid in the prevention of coccidiosis caused by *Eimeria necatrix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*.
[9]Optiphos® PF 2000- Huvepharma, Peachtree City, GA

Table III-2. Ingredient profile and calculated nutrient concentration of reduced-energy diets (finisher phase) containing corn screenings fed to male broilers.

	Finisher			
Nutrient	**HE[1]**	**HE+CS[3]**	**LE[2]**	**LE+CS[3]**
Corn	66.77	44.45	69.46	47.24
Dehulled Soybean Meal (48%)	21.72	23.00	21.23	22.49
DL-Methionine (99%)	0.21	0.20	0.20	0.20
Lysine HCL	0.19	0.15	0.20	0.16
Fat, Blended	2.75	3.88	0.50	1.59
Calcium Carbonate	1.13	1.09	1.14	1.10
Monocalcium Phosphate	0.38	0.39	0.38	0.38
Salt	0.19	0.20	0.23	0.20
Sodium Bicarbonate	0.27	0.25	0.28	0.26
Trace Minerals[4,5,6]	0.05	0.05	0.05	0.05
Vitamins[7]	0.25	0.25	0.25	0.25
Salinomycin[8]	0.05	0.05	0.50	0.05
DDGS	5.00	5.00	5.00	5.00
Phytase[9]	0.013	0.013	0.013	0.013
Corn Screenings	0.00	20.00	0.00	20.00
Meat and Bone Meal	1.00	1.00	1.00	1.00
Calculated Nutrient Concentration				
Protein	18.50	18.50	18.50	18.50
Lysine	1.02	1.02	1.02	1.02
Methionine	0.50	0.49	0.50	0.49
TSAA	0.81	0.80	0.81	0.80
Threonine	0.20	0.70	0.71	0.70
Calcium	0.75	0.75	0.75	0.75
Available Phosphorus	0.36	0.36	0.36	0.36
Total Phosphorus	0.48	0.46	0.48	0.47
Sodium	0.18	0.18	0.20	0.18
Metabolizable Energy (kcal/kg)	3157	3157	3047	3047

[1]High Energy (HE) - Standard corn/soybean meal diet; [2]Low Energy (LE)-Energy reduced by 110kcal/kg compared to HE

[3]Corn screenings were included at 20% in the diet

[4] Trace mineral premix added in the starter yields 180 mg of manganese, 108 mg of total zinc, 5.1 mg of copper, 3.51 mg of iodine, 0.3 mg of total selenium, 0.013 g of *bacillus subtilis*

[5] Trace mineral premix added in the grower yields 150 mg of manganese, 90 mg of total zinc, 4.25 mg of copper, 2.92 mg of iodine, 0.25 mg of total selenium, 0.011 g of *bacillus subtilis*

[6] Trace mineral premix added in the finisher yields 90 mg of manganese, 54 mg of total zinc, 2.55 mg of copper, 1.75 mg of iodine, 0.15 mg of total selenium, 0.007 g of *bacillus subtilis*

[7] Vitamin premix added at this rate yields 7,700 IU vitamin A, 5,500 ICU vitamin D_3, 55 IU vitamin E, 1.5 mg vitamin K-3, 0.01 mg B_{12}, 6.6 mg riboflavin, 38.5 mg niacin, 9.9 mg d-pantothenic acid, 0.88 mg folic acid, 2.75 mg pyroxidine, 1.54 mg thiamine, 0.08 mg biotin per kg diet

[8]Active drug ingredient salinomycin sodium, 60 g/lb. (60 g/ton inclusion; Huvepharma, St. Louis, MO). As an aid in the prevention of coccidiosis caused by *Eimeria necatrix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*.

[9]Optiphos® PF 2000- Huvepharma, Peachtree City, GA

Table III-3. Analyzed nutrient content[4] of reduced-energy diets (starter and grower phase) containing corn screenings fed to male broilers.

	Starter				Grower			
Treatment	**HE[1]**	**HE+CS[3]**	**LE[2]**	**LE+CS[3]**	**HE[1]**	**HE+CS[3]**	**LE[2]**	**LE+CS[3]**
Moisture (%)	13.00	12.70	13.00	13.10	12.10	12.40	11.90	12.10
Dry matter (%)	87.00	87.30	87.00	86.90	87.90	87.60	88.10	87.80
Protein (crude) (%)	21.60	23.40	22.20	21.60	20.30	21.30	20.70	20.20
Fat (crude) (%)	6.24	6.92	4.63	5.20	5.63	6.43	3.94	4.39
Ash (%)	5.60	5.60	5.20	5.40	5.00	4.80	4.70	5.40
Starch (%)	35.40	32.50	38.60	35.30	39.50	37.60	40.50	38.80
Sulfur (total) (%)	0.34	0.33	0.33	0.31	0.28	0.29	0.29	0.29
Phosphorus (total) (%)	0.67	0.67	0.64	0.64	0.55	0.58	0.61	0.60
Potassium (total) (%)	1.01	1.06	0.95	1.08	0.90	0.92	0.98	0.94
Magnesium (total) (%)	0.17	0.17	0.16	0.18	0.15	0.16	0.16	0.16
Calcium (total) (%)	1.11	1.07	1.08	1.00	0.92	0.92	0.98	1.17
Sodium (total) (%)	0.21	0.19	0.21	0.19	0.20	0.20	0.21	0.21
Iron (total) ppm	264	236	224	213	183	231	214	204
Manganese (total) ppm	88	77	90	85	71	70	78	80
Copper (total) ppm	14	12	14	14	13	14	12	14
Zinc (total) ppm	92	84	99	84	77	83	84	84

[1]High Energy (HE) - Standard corn/soybean meal diet; [2]Low Energy (LE)-Energy reduced by 110kcal/kg compared to HE

[3]Corn screenings (CS) were included at 20% in the diet

[4]Analyzed nutrient content conducted by Midwest Laboratories, Omaha, NE

[5]Analyzed xylanase recovery was 208, 183, and 196 for the control diet and 226, 307, and 196 for the diet containing corn screenings for the starter, grower and finisher phases, respectively

Table III-4. Analyzed nutrient content[4] of reduced-energy diets (finisher phase) containing corn screenings fed to male broilers.

	Finisher				Corn Screenings
Treatment	**HE[1]**	**HE+CS[3]**	**LE[2]**	**CS+ LE[3]**	
Moisture (%)	12.35	12.47	11.56	12.13	14.30
Dry matter (%)	87.65	87.53	88.44	87.87	85.70
Protein (crude) (%)	18.50	17.90	18.10	18.80	9.86
Fat (crude) (%)	5.60	6.49	3.71	4.39	2.92
Ash (%)	4.00	4.43	4.32	4.03	1.30
Starch (%)	42.50	40.17	43.10	41.67	59.20
Sulfur (total) (%)	0.24	0.25	0.26	0.25	0.09
Phosphorus (total) (%)	0.50	0.51	0.54	0.54	0.22
Potassium (total) (%)	0.81	0.83	0.81	0.88	0.33
Magnesium (total) (%)	0.13	0.14	0.13	0.14	0.08
Calcium (total) (%)	0.70	0.76	0.80	0.69	0.03
Sodium (total) (%)	0.15	0.18	0.20	0.15	0.00
Iron (total) ppm	274	188	185	183	72.00
Manganese (total) ppm	70	76	71	70	7
Copper (total) ppm	11	12	11	10	3
Zinc (total) ppm	94	89	83	71	19

[1]High Energy (HE) - Standard corn/soybean meal diet; [2]Low Energy (LE)-Energy reduced by 110kcal/kg compared to HE
[3]Corn screenings (CS) were included at 20% in the diet
[4]Analyzed nutrient content conducted by Midwest Laboratories, Omaha, NE

Statistical Analysis

All data were subjected to a 2 X 3 Factorial Analysis of Variance (**ANOVA**) using SPSS V 22.0 with main effect means being statistically different at a $P \leq 0.05$. In the instance of a significant interaction present between corn type and enzyme, data were subjected to a one way ANOVA and individual treatment means separated by Duncan's multiple range test.

Results

Body Weight (BW). On d 14, diet type influenced BW with birds being fed without corn screenings having a higher ($P < 0.05$) BW compared to diets with 20% corn screenings (Table III-5). On d 27, a significant interaction was observed between diet and energy. Although no differences were observed between the HE and LE in the control diet, a reduction ($P < 0.05$) in BW was observed in the diet containing corn screenings when comparing the LE to the HE. While the inclusion of NSPase in the control diet did not influence BW, NSPase supplementation in the LE diet containing corn screenings increased BW to levels similar to that of the high energy. On d 42, no significant differences were observed amongst treatments in relation to average BW.

Table III-5. Body weight (BW) of male broilers fed reduced energy diets containing corn screenings.

		Body Weight (kg)			
Diet	**Enzyme**	d 0	d 14	d 27	d 42
Control	High Energy[1]	0.042	0.412	1.493[ab]	2.620
Control	Low Energy[2]	0.043	0.413	1.487[ab]	2.666
Control	Low Energy + NSPase[3]	0.043	0.412	1.500[a]	2.707
Corn Screenings	High Energy[1]	0.043	0.401	1.472[ab]	2.677
Corn Screenings	Low Energy[2]	0.043	0.399	1.404[c]	2.664
Corn Screenings	Low Energy + NSPase[3]	0.043	0.409	1.461[b]	2.606
Main Effects					
Diet					
Corn Screenings		0.043	0.403[b]	1.446	2.649
Control		0.043	0.412[a]	1.494	2.664
Energy					
High Energy (HE)[1]		0.042	0.407	1.483	2.649
Low Energy (LE)[2]		0.043	0.406	1.445	2.665
LE + NSPase (LEN)[3]		0.043	0.410	1.481	2.656
P-value					
Diet		0.436	0.003	<0.001	0.870
Energy		0.053	0.339	0.003	0.998
Diet X Energy		0.700	0.294	0.027	0.143
Pooled SEM		0.082	0.002	0.006	0.023

[a-c] Means within a column with different superscripts differ at $P < 0.05$

[1]High Energy (HE)- Standard corn/soybean meal diet

[2]Low Energy (LE)-Energy reduced by 110kcal/kg compared to HE

[3]NSPase-Inclusion of 113.5 g/ton (Enspira®; United Animal Health, Sheridan, IN)

Mortality Adjusted Feed Conversion Ratio (FCR). Mortality corrected feed conversion ratio was consistently impacted with the variations of energy in the diet and the presence of NSPase. During the starter phase, the presence of corn screenings did not impact observed FCR, however, the reduction in energy in the LE diet increased ($P < 0.05$) FCR compared to the HE diet (Table III-6). The inclusion of NSPase did not

impact starter diet FCR. During the grower period, diet impacted FCR with birds being fed corn screenings exhibiting a higher ($P < 0.05$) FCR compared to diets absent of screenings. Similar to the starter period, reduction of dietary energy in the LE diet negatively impacted FCR as broilers fed the LE diet had an increased ($P < 0.05$) FCR compared to the HE fed broilers. The addition of NSPase in the LEN treatment during the grower phase reduced ($P < 0.05$) FCR compared to the LE fed broilers. Similar results were observed for the cumulative FCR from d 1 to 27 with corn screenings increasing ($P < 0.05$) FCR, energy reduction increasing ($P < 0.05$) FCR, and NSPase decreasing ($P < 0.05$) FCR. During the finisher phase, diet influenced FCR as broilers fed diets including corn screenings had a lower ($P < 0.05$) FCR. At the conclusion of the trial (d1-42), the presence of corn screenings did not impact FCR. Reduction of dietary energy increased ($P < 0.05$) FCR as compared to the positive control (PC) fed broilers. Inclusion of the NSPase in the LEN diet improved ($P < 0.05$) observed FCR (d 1 to 42) as compared to the LE diet and were similar to the HE fed broilers.

Table III-6. Feed Conversion Ratio (FCR) of male broilers fed reduced energy diets containing corn screenings.

		Feed Conversion Ratio (feed:gain)				
Diet	**Enzyme**	Starter	Grower	d1-27	Finisher	d1-42
Control	High Energy[1]	1.182	1.471	1.396	2.114	1.701
Control	Low Energy[2]	1.214	1.499	1.425	2.162	1.747
Control	Low Energy + NSPase[3]	1.212	1.491	1.419	2.076	1.706
Corn Screenings	High Energy[1]	1.202	1.501	1.425	2.052	1.703
Corn Screenings	Low Energy[2]	1.221	1.572	1.479	2.032	1.736
Corn Screenings	Low Energy + NSPase[3]	1.206	1.528	1.443	2.101	1.727
Main Effects						
Diet						
Corn Screenings		1.209	1.534[a]	1.449[a]	2.062[b]	1.722
Control		1.202	1.487[b]	1.413[b]	2.118[a]	1.718
Energy						
High Energy (HE)[1]		1.192[b]	1.486[c]	1.411[c]	2.083	1.702[b]
Low Energy (LE)[2]		1.217[a]	1.535[a]	1.452[a]	2.097	1.741[a]
LE + NSPase (LEN)[3]		1.209[a]	1.510[b]	1.431[b]	2.089	1.716[b]
P-value						
Diet		0.156	<0.001	<0.001	0.022	0.802
Energy		0.002	<0.001	<0.001	0.682	0.001
Diet X Energy		0.212	0.123	0.138	0.069	0.329
Pooled SEM		0.003	0.006	0.004	0.017	0.006

[a-c] Means within a column with different superscripts differ at $P < 0.05$

[1]High Energy (HE)- Standard corn/soybean meal diet

[2]Low Energy (LE)-Energy reduced by 110kcal/kg compared to HE

[3]NSPase-Inclusion of 113.5 g/ton (Enspira®; United Animal Health, Sheridan, IN)

Feed Consumption (FC) and Mortality. During the starter phase, diet influenced FC with the birds being fed corn screenings exhibiting a reduction ($P < 0.05$) in FC compared to the control diets (Table III-7) which contributed to the reduced BW observed at d 14. Birds fed the low energy diet did not observe differences in FC when

compared to the HE diet, however, the LEN diet increased ($P < 0.05$) FC when compared to the HE. Following the starter phase, neither diet nor energy level influenced FC for the duration of the trial. No differences in mortality were observed among treatments throughout the study (Table III-8).

Table III-7. Feed consumption (FC) of male broilers fed reduced energy diets containing corn screenings.

		Feed Consumption (g/bird/day)				
Diet	**Enzyme**	[4]Starter	[5]Grower	d1-27	[6]Finisher	d1-42
Control	High Energy[1]	33.2	113.2	74.2	159.8	104.2
Control	Low Energy[2]	34.2	114.2	75.3	166.3	107.3
Control	Low Energy + NSPase[3]	34.0	115.5	75.9	165.2	107.3
Corn Screenings	High Energy[1]	32.9	114.6	75.0	162.0	105.8
Corn Screenings	Low Energy[2]	33.0	112.0	73.5	167.0	106.2
Corn Screenings	Low Energy + NSPase[3]	33.7	113.9	75.0	157.4	104.0
Main Effects						
Diet						
Corn Screenings		33.2[b]	113.5	74.5	162.2	105.3
Control		33.8[a]	114.3	75.1	163.8	106.2
Enzyme						
High Energy (HE)[1]		33.0[b]	113.9	74.6	160.9	105.0
Low Energy (LE)[2]		33.6[ab]	113.1	74.4	166.7	106.8
LE + NSPase (LEN)[3]		33.9[a]	114.7	75.5	161.3	105.6
P-value						
Diet		0.024	0.253	0.128	0.579	0.432
Energy		0.026	0.208	0.094	0.171	0.402
Diet X Energy		0.193	0.107	0.053	0.300	0.198
Pooled SEM		0.100	0.400	0.300	1.700	0.700

[a-b] Means within a column with different superscripts differ at $P < 0.05$

[1]High Energy (HE)- Standard corn/soybean meal diet

[2]Low Energy (LE)-Energy reduced by 110kcal/kg compared to HE

[3]NSPase-Inclusion of 113.5 g/ton (Enspira®; United Animal Health, Sheridan, IN)

[4]Starter Phase: d 0-14, [5]Grower Phase: d 15-27, [6]Finisher Phase: d 28-42

Table III-8. Mortality (%) of male broilers fed reduced energy diets containing corn screenings.

		Mortality (%)			
Diet	**Enzyme**	[4]Starter	[5]Grower	[6]Finisher	d1-42
Control	High Energy[1]	2.50	0.010	2.00	5.30
Control	Low Energy[2]	1.90	0.006	1.30	3.80
Control	Low Energy + NSPase[3]	3.50	0.007	2.30	6.30
Corn Screenings	High Energy[1]	1.60	0.003	1.30	3.10
Corn Screenings	Low Energy[2]	2.80	0.004	0.60	3.80
Corn Screenings	Low Energy + NSPase[3]	0.60	0.009	2.30	3.70
Main Effects					
Diet					
Corn Screenings		1.70	0.005	1.40	3.50
Control		2.60	0.008	1.90	5.10
Enzyme					
High Energy (HE)[1]		2.00	0.006	1.60	4.20
Low Energy (LE)[2]		2.30	0.005	1.00	3.80
LE + NSPase (LEN)[3]		2.00	0.008	2.30	5.00
P-value					
Diet		0.198	0.534	0.401	0.095
Energy		0.915	0.788	0.133	0.562
Diet X Energy		0.117	0.565	0.825	0.485
Pooled SEM		0.003	0.002	0.003	0.005

[a-b] Means within a column with different superscripts differ at $P < 0.05$

[1]High Energy (HE)- Standard corn/soybean meal diet

[2]Low Energy (LE)-Energy reduced by 110kcal/kg compared to HE

[3]NSPase-Inclusion of 113.5 g/ton (Enspira®; United Animal Health, Sheridan, IN)

[4]Starter Phase: d 0-14, [5]Grower Phase: d 15-27, [6]Finisher Phase: d 28-42

Discussion

Feed costs account for approximately 70 % of total poultry production costs and are continually increasing due to the rising demand of cereal grains for biofuel production and world consumption (Donohue and Cunningham, 2009). Alternative feed

ingredients such as grain by-products (DDGS, wheat middlings, corn screenings etc.) have become a viable option in reducing diet costs as they are cheaper and less desirable compared to traditional feed ingredients (Campasino et al., 2015). It should be noted that grain by-products vary greatly in nutrient content and are typically higher in fiber concentration (NRC, 1994; Widyaratne and Zijlstra, 2007). Elevator configuration and cleaner operating practices have been known to be primary contributors to the variation in the nutritional content of screenings (Meinders, 1993). Preliminary research observed higher fiber and ash (1 to 2%) values in small fines when evaluating screening composition (Hill and Garcia., 1982). It is suggested that the higher fiber content and lower energy value associated with corn screenings can be related to the greater portion of cell wall components and a smaller portion of endosperm. It is presumed that the reduction of endosperm content would result in a lower starch value as starch is the major storage carbohydrate of cereals and is located within the endosperm in granular form (Koehler and Wieser, 2013). Furthermore, the increased cell wall components could attribute to the higher fiber value as well as an increase in NSP and substrate content. While previous research involving the use of corn screenings in poultry diets is minimal, this corn-by product could potentially be used as an alternative feed ingredient for poultry producers.

In the current trial, the inclusion of corn screenings reduced broiler BW when compared to control diets on d 14. This may be due to the increased fiber content found within screenings as fiber has been known to negatively affect chick growth (Janssen and Carre, 1985). It is possible that the higher fiber content found within corn screenings

may have negatively impacted digestion as there was a decrease in FC which resulted in lighter bird weights during the starter phase. While FC was reduced early in the trial, no further differences in consumption were observed following the starter phase. It has been reported that the response to increases in dietary fiber varies depending on a multitude of factors including level and type of fiber, bird age, and composition of diet (González-Alvarado et al., 2010; González-Alvarado et al., 2008; Hetland et al., 2003; Jiménez-Moreno et al., 2016). Mazhari et al. (2011) stated that birds fed wheat screenings at 24% resulted in a 15% lower BW gain compared to the control diet. Furthermore, Stapleton et al. (1980) observed a reduction in BW when incorporating wheat screenings at 60% in diets compared to a standard commercial diet through four weeks of age. Although the reduction of BW in the current trial was attributed to the reduction in FC, it may also be associated with an increased rate of feed passage resulting in less efficient digestion and absorption of nutrients (Rochell et al., 2012). Ravindran et al. (1984) noted an increase in rate of passage in swine when increasing insoluble fiber content. These results indicate that fiber content could possibly influence passage rate in broilers. The significant interaction between diet and enzyme on d 27 could be related to the increased fiber and substrate content of the corn screenings. It is possible that the inclusion of corn screenings in the diet allowed for a greater enzyme response due to the increased substrate content. Ravindran (2013) stated that the reaction rate of enzymes increases as the concentration of substrate in the diet increases. Furthermore, Meng et al. (2005) reported that carbohydrases are able to degrade cell wall polysaccharides releasing encapsulated nutrients such as starch and amino acids. This may explain the increase in

BW on d 27 when supplementing NSPase in the low energy (-110 kcal/kg) diet containing corn screenings. Cowieson et al. (2010) noted that approximately 400 to 450 kcal of energy is not digested in corn-soy diets allowing for improvement through enzyme supplementation. It is hypothesized that the inclusion of NSPase in the diet elicited an effect by hydrolyzing the beta-bonds releasing sufficient amounts of energy and protein that was used for muscle growth which resulted in an overall increase in weight gain when compared to the energy deficient diet without enzyme inclusion. Similar results were observed by Cowieson et al. (2010) in which supplementing reduced energy (-110kcal/kg) diets with a combination of xylanase and glucanase at 8,000 and 15,000 U/ kg respectively, increased BW by 4.5% compared to the NC diet. In order to successfully incorporate corn screenings in commercial diets, accurate nutrient profiles of corn screenings are necessary for effectively utilizing this grain by-product. Hess et al. (1999) reported nutrient levels and the presence of mycotoxins and anti-nutritional factors varying more for corn screenings more than other feed ingredients. Due to the varying nutrient content within screenings, broiler performance could be negatively impacted if nutrient profiles obtained from whole corn were used instead of corn screening values.

Diet composition did not impact FCR during the starter phase. While the main effect of energy level resulted in no differences in starter FCR in the LEN when compared to the LE diet, the removal of energy from the diet resulted in a significant increase in FCR when compared to the HE for the starter period. Williams et al. (2014a)

observed similar results during the starter phase with a 15 point increase in FCR occurring in the reduced energy (-88 kcal/kg) diet compared to a standard corn-soybean meal diet. In the grower phase and cumulative through d 27 of the current trial, the inclusion of corn screenings increased FCR when compared to the control diets. This could be attributed to the anti-nutritive properties associated with NSP in corn screenings exerting a negative impact on FCR resulting in poorer feed conversion when compared to birds fed the control diets. While intestinal viscosity was not measured in the current study, it could have possibly been one of the mechanisms involved in influencing performance as increased gut viscosity can inhibit digestion and absorption of nutrients, reduce the diffusion of endogenous enzymes, as well as alter gut microbiology (Langhout, 1998; Smits and Annison, 1996). Mazhari et al. (2015) observed an increase in intestinal viscosity in broilers fed varying levels of wheat screenings compared to diets without screenings. The supplementation of NSPase in the current study reduced FCR during the grower phase and cumulative through d 27 when compared to the energy-deficient diet. It is believed that the supplementation of NSPase in the diet could have reduced gut viscosity and enabled access to the encapsulated nutrients within the cell walls resulting in an improved FCR. With regards to the finisher phase, there was an inverse trend as birds fed the diet containing screenings resulted in lower FCR compared to birds fed the control diets. While this outcome was unexpected and unclear, it is hypothesized that the greater proportion of fiber in the corn screenings influenced gut microbiota concentrations. Barnes et al. (1972) reported that the intestinal microflora of chickens was altered over a period of 2 to 6 weeks of age indicating that

the increased fiber content in the corn screenings could have reformed the gut microbiota eliciting a beneficial effect in the finisher phase. Previous research has suggested that that fermentation of NSP from gut microbiota results in the increased production of short chain fatty acids (SCFA) which could then be used as a potential energy source (McWhorter et al., 2009; Sergeant et al., 2014). While no differences were observed in diet composition for cumulative d 1 to 42, the main effect of energy level on FCR was significant with the energy-deficient diet increasing FCR by 4 points when compared to the HE diet. O'Neill et al. (2012b) observed similar results when evaluating xylanase efficacy in reduced energy diets with and without fat compared to standard corn-soybean meal diets. The reduction of energy (-100 kcal/kg) in both the NC diets with or without fat increased FCR by 7 and 8 points respectively, when compared to the PC for the cumulative phase (d 1 to 42). With regards to the LEN diet, FCR was improved to levels that were similar to the HE implying that NSPase supplementation can recover caloric values removed in the LE diet. Research conducted by Cowieson et al. (2010) agree with this data as cumulative FCR was improved to levels comparable to the PC when supplementing both xylanase and glucanase in low-energy diets at 8,000 and 15,00 U/kg respectively.

Feed consumption was impacted during the starter phase with the inclusion of corn screenings reducing FC compared to the control. Leeson et al. (1991) observed similar results from d 11 to 21 with a reduction in FC in male broilers fed diets diluted with 25% ground rice hulls. While no differences were observed between the HE and LE

diets, the inclusion of NSPase in the LEN diet significantly increased FC by 1 g when compared to the HE. Coppedge et al. (2012) also reported no differences in FC during the starter phase when comparing reduced energy (-133 kcal/kg) diets to standard corn-soybean diets. However, in contrast to the current trial, NSPase inclusion did not influence starter FC when compared to the high energy diet. Throughout the remainder of the current study, main effects for diet and energy level did not affect FC. This data agrees with research conducted by Mazhari et al. (2015) in which diets fed wheat screenings at levels of 150, 300, 450, and 600 g/kg did not impact FC when compared to the control diet. Furthermore, both Saki and Alipana (2005) and Audren et al. (2002) observed no effect on FC when feeding diets with varying levels of wheat screenings.

In the present study, the incorporation of corn screenings at 20% suppressed early growth performance, however, no differences between diet types were observed at the conclusion of the trial indicating corn screenings can be successfully incorporated in broiler diets. Furthermore, supplementing NSPase in corn-soybean meal diets with and without corn screenings improved performance parameters of male broilers in both diet types. The results of this study indicate that the supplementation of NSPase can recover the caloric value lost in low energy diets as well mitigate the anti-nutritive factors associated with NSP in corn screenings. These data suggests that removing energy from the diet as well as incorporating lower quality ingredients such as corn screenings can be potential feeding strategies for poultry producers.

CHAPTER IV

EVALUATION OF COCKTAIL NSPASE INCLUSION IN REDUCED-ENERGY CORN-SOYBEAN MEAL DIETS ON LIVE GROWTH PERFORMANCE AND CARCASS YIELD OF MALE BROILERS[*]

Introduction

Biofuels derived from grains such as corn have substantially increased in the U.S. with production expanding from 2 billion gallons in 2001 to 14 billion gallons in 2011 (Locke et al., 2013). This alternative fuel source has increased in popularity as it is a cleaner, more renewable energy source than conventional fossil fuels (Lumpkins et al., 2004). Most ethanol production derives from corn, which is the most abundant crop in the U.S., resulting in the redirection of its use away from livestock production. This change has led to further increases in the price of corn for use in livestock and poultry feed ingredients (Donohue and Cunningham, 2009) as well as a greater presence of potential by-product meal sources for dietary inclusion. Distillers dried grains with solubles **(**DDGS) are the byproduct of corn fermentation in the production of ethanol and have been incorporated in poultry diets at an inclusion rate of 2.5 to 5% as an alternative protein source (Lumpkins et al., 2004). While DDGS generally contain greater

[*] Reprinted from "Evaluation of Cocktail NSPase Inclusion in Reduced-Energy Corn-soybean Meal Diets on Live Growth Performance and Carcass Yield of Male Broilers" by H.G. Walters, M. P. Williams, R. Latham, N. Augspurger, B. Brown, and J. T. Lee. 2018. International Journal of Poultry Science, 17, 610-619. Copyright [2018] by H.G. Walters et al.

concentrations of protein, fat, vitamins, and minerals when compared to whole kernel corn, several nutritional disadvantages limit higher inclusion levels (Lim and Yildirim-Aksoy, 2008). Nutritional variability, NSP content, and physical quality of the diet become a concern when feeding DDGS (Loar et al., 2012; Pedersen et al., 2014). The total NSP content of DDGS is approximately 4 times that of whole kernel corn, with the main constituents being 16% cellulose, 8% xylans, and 5% arabinans (Kim et al., 2008; Pedersen et al., 2014). It is often speculated that the higher NSP content and nutrient variability associated with DDGS may impede digestion and nutrient absorption, resulting in a negative impact on bird performance. The use of exogenous enzymes is a potential strategy to reduce the deleterious effects of NSP and by-product inclusion by improving the nutrient utilization of DDGS when included in corn- and soybean meal-based diets.

In the U.S., corn and soybean meal are the two major feed ingredients used in poultry diets. While the NSP content of these feedstuffs is low compared to that of DDGS, their contribution to the overall NSP content of the diet is considerable due to their high inclusion rate (Yegani and Korver, 2013). Approximately 90% of plant cell walls are composed of NSP, which sequester vital nutrients such as starch, proteins, and lipids. These fibrous biopolymers are linked by glycosidic bonds within the cell wall and surround the starchy endosperm and aleurone layers, interfering with nutrient digestibility and phytate dephosphorylation (Caprita et al., 2010; Slominski, 2011). Corn and soybean meal contain approximately 10 and 20% NSP, respectively, according to CVB (1998). Common NSP found in SBM and corn consists of arabinose, xylose,

raffinose, rhamnose, and galactose (Coppedge et al., 2012). These NSP are known to possess antinutritional properties that decrease nutrient digestibility, prevent access to nutrients through encapsulation, and reduce broiler performance (Williams et al., 1997).

To ameliorate the negative effect of NSP, exogenous enzymes have been introduced to improve performance by hydrolyzing indigestible bonds in the plant cell walls into smaller fragments, allowing increased digestibility and improved bird performance (Coppedge et al., 2012). It is hypothesized that while a single enzyme acts on a certain substrate, cocktail carbohydrases allow multiple enzymes to act on various substrates, improving nutrient digestion and absorption. Previous research has shown that carbohydrase products improve weight gain and feed conversion ratio (FCR) in broilers fed corn and soybean meal diets due to the increased ileal digestibility of protein and NSP (Marsman et al., 1997; Zanella et al., 1999). The objective of this study was to evaluate the impact of a cocktail NSPase in low-energy diets containing DDGS on broiler performance and processing parameters.

Materials and Methods

Experimental Design

Experiment 1 and Experiment 2. On the day of hatch, 1,050 Cobb 500 male broiler chicks were allotted to floor pens and dietary treatments based on initial BW. Each treatment consisted of 10 replicates containing 35 birds per replicate pen. Chicks were provided age-appropriate supplemental heat and given access to feed and water *ad libitum.* Chicks were placed in 1.83 m x 1.83 m rearing pens equipped with tube feeders and nipple drinkers with recycled litter as bedding material. Animal care was provided in

accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC). The dietary program for experiment 1 consisted of 3 dietary phases with a starter diet being fed from 1 to 14 d of age, grower from 15 to 28 d of age and finisher from 29 to 42 d. Experiment 2 consisted of 4 dietary phases including a starter diet from 1 to 14 d of age, grower from 15 to 28 d, a finisher diet from 29 to 35 d, and a withdrawal from 36 to 42 d. All broilers and feed were weighed on the days of dietary changes for determination of average body weight (BW) and feed consumption (FC) for the calculation of mortality-corrected feed conversion ratio (FCR). Upon completion of each trial (d 42), 6 broilers per replicate were randomly selected and processed to obtain processing yield data including carcass without giblet (WOG) and fat pad weight and yield. All broilers were bulk weighed on the evening of d 42 prior to an 8 h feed withdrawal period for processing on d 43. Six broilers from each replicate pen (60 broilers/treatment) were removed and individually weighed before processing. Carcass weight (WOG) and fat pad weights were determined and yields were calculated following processing prior to emerging chilling.

Experiment 3. On the day of hatch, 900 Cobb 500 male broiler chicks were allotted to floor pens and dietary treatments based on initial BW. Each treatment included 10 replicates containing 30 birds per replicate pen. Chicks were provided age-appropriate supplemental heat and given access to feed and water *ad libitum.* Chicks were placed in 1.83 m x 1.52 m rearing pens equipped with tube feeders and nipple drinkers with recycled litter as bedding material. Animal care was provided in accordance with a protocol approved by the Institutional Animal Care and Use

Committee (IACUC). The dietary program consisted of 3 dietary phases including a starter diet from 1 to 14 d of age, a grower diet from 15 to 28 d, and a finisher diet being fed from 29 to 42 d of age. All broilers and feed were weighed on the days of dietary changes for determination of average BW and FC for the calculation of mortality-corrected FCR.

Experimental Diets

The effect of NSPase[1] (cocktail carbohydrase) inclusion on broiler growth performance and processing yields in reduced-energy diets was evaluated in three consecutive trials with a completely randomized experimental block design containing 3 dietary treatments during a 42 d grow-out. The dietary treatments included a positive control (PC), negative control (NC) with a 132 kcal/kg ME reduction compared to the PC, and the NC supplemented with NSPase. Diets were corn and soybean meal-based with a 5% DDGS inclusion throughout the trial for experiment 1 and experiment 3. Experiment 1 included pork meat and bone meal at an inclusion rate of 3% for the starter, grower, and finisher diets. Experiment 2 consisted of 5% DDGS inclusion during the starter phase with a 10% inclusion of DDGS for the remainder of the trial. PC diets were formulated to amino acid and energy levels of that found in a typical industry diet (Tables IV-1, IV-2 and IV-3). During feed manufacturing, treatments 2 and 3 were mixed as one large basal diet and divided equally prior to enzyme inclusion. All diets

[1] Enspira®, United Animal Health- Sheridan, IN; 2,700 U/g of xylanase from *Aspergillus niger* and *Trichoderma reesei*; also contains ß-glucanase and α-galactosidase

contained 250 FTU/kg of phytase[2]. The NSPase was included at a rate of 113.5 g/ton (2,700 U/g of xylanase from *Aspergillus niger* and *Trichoderma reesei*; also contains ß-glucanase and α-galactosidase) for all dietary phases with recovery analysis in the footnote of each diet table. All diets were pelleted with the exception of the starter diet, which was pelleted and then crumbled. Diets were pelleted at a temperature range of 80 to 85°C with a conditioning time of approximately 12 seconds. Samples were collected in duplicate during feed manufacturing for nutrient analysis. Crude protein was determined using AOAC by combustion (AOAC 990.03), total P was determined by wet ash ICP (AOAC 985.01M), acid detergent fiber was determined using an ANKOM digestion unit (AOAC 973.18), and ether extraction was used to determine crude fat (AOAC 920.39).

[2] OptiPhos® 2000 PF, Huvepharma, St. Louis, MO

Table IV-1. Dietary formulations and calculated and analyzed nutrient content of the Positive Control (PC) and Negative Control (NC) fed to male market broilers in experiment 1.

	Starter		Grower		Finisher	
Ingredient (%)	**PC**	**NC**	**PC**	**NC**	**PC**	**NC**
Corn	56.03	60.48	62.33	64.82	67.47	66.83
Dehulled Soybean Meal (48%)	29.88	28.28	24.70	24.39	19.82	22.79
DL-Methionine (99%)	0.26	0.27	0.24	0.24	0.16	0.15
Dried Distillers Grain	5.00	5.00	5.00	5.00	5.00	5.00
Lysine HCL	0.23	0.27	0.21	0.22	0.19	0.09
Fat, A/V Blend	3.42	0.50	2.69	0.50	2.63	0.50
Limestone	1.02	1.03	0.83	0.83	0.75	0.74
Monocalcium Phosphate	0.39	0.39	0.18	0.17	0.11	0.09
Sodium Chloride	0.41	0.37	0.29	0.29	0.18	0.29
Sodium Bicarbonate	0.01	0.06	0.17	0.18	0.33	0.17
Vitamin Premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Trace Minerals[2]	0.05	0.05	0.05	0.05	0.05	0.05
Coban 90[3]	0.05	0.05	0.05	0.05	0.05	0.05
Phytase[4]	0.01	0.01	0.01	0.01	0.01	0.01
Pork Meat and Bone Meal	3.00	3.00	3.00	3.00	3.00	3.00
Calculated Nutrient Content						
Protein	22.50	22.45	20.50	20.50	18.50	19.78
dig-Lysine	1.18	1.18	1.04	1.04	0.90	0.90
dig-Methionine	0.57	0.57	0.53	0.53	0.43	0.43
dig-TSAA	0.85	0.85	0.80	0.80	0.67	0.68
dig-Threonine	0.70	0.69	0.64	0.64	0.56	0.61
Calcium	0.92	0.92	0.80	0.80	0.75	0.75
Available Phosphorus	0.45	0.45	0.40	0.40	0.38	0.38
Total Phosphorus	0.57	0.57	0.51	0.52	0.48	0.50
Sodium	0.20	0.20	0.20	0.20	0.20	0.20
Apparent Metabolizable Energy	3102	2970	3124	2992	3168	3036
Analyzed Nutrient Content[5]						
Crude Protein	22.20	22.70	21.70	20.60	17.20	18.80
Crude Fat	4.93	3.49	5.30	2.90	5.09	3.45
Total Phosphorous	0.66	0.65	0.67	0.58	0.49	0.55
Acid Detergent Fiber	4.20	4.30	3.70	3.10	2.90	2.80

[1] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, and 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[2] Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate, and the premix contains less than 1% mineral oil.

[3] Active drug ingredient monensin sodium, 90 g/lb (90 g/ton inclusion; Elanco Animal Health, Indianapolis, IN), as an aid in the prevention of coccidiosis caused by *Eimeria necatrix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*.

[4] Optiphos. Huvepharma- St. Louis, MO.

[5] Analyzed enzyme recovery was 238 U/kg for starter, 260 U/kg for grower, and 193 U/kg for the finisher.

Table IV-2. Dietary formulations and calculated and analyzed nutrient content of the Positive Control (PC) and Negative Control (NC) fed to male market broilers in experiment 2.

Ingredient	Starter (%)		Grower (%)		Finisher (%)		Withdrawal	
	PC	NC	PC	NC	PC	NC	PC	NC
Corn	52.63	55.96	55.70	57.84	58.75	62.25	65.89	69.01
Dehulled Soybean Meal (48%)	35.22	34.62	28.12	27.74	25.28	24.45	18.52	18.11
DL-Methionine (99%)	0.26	0.25	0.20	0.20	0.10	0.10	0.14	0.13
Dried Distillers Grain	5.00	5.00	10.00	10.00	10.00	10.00	10.00	10.00
Lysine HCL	0.14	0.15	0.12	0.21	0.05	0.07	0.22	0.22
Fat, A/V Blend	3.25	0.50	2.83	0.50	3.25	0.50	2.82	0.11
Limestone	1.85	1.86	1.51	1.52	1.30	1.31	1.16	1.17
Sodium Bicarbonate	0.00	0.00	0.00	0.00	0.01	0.03	0.32	0.32
Monocalcium Phosphate	0.86	0.86	0.71	0.71	0.54	0.54	0.49	0.48
Sodium Chloride	0.44	0.44	0.37	0.42	0.36	0.40	0.14	0.14
Vitamin Premix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace Minerals[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Coban 90[3]	0.05	0.05	0.05	0.05	0.05	0.05	0.00	0.00
Phytase[4]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Calculated Nutrient Content								
Protein	22.10	22.15	21.33	21.37	19.97	19.88	17.47	17.54
dig-Lysine	1.18	1.18	1.07	1.07	0.88	0.88	0.85	0.85
dig-Methionine	0.60	0.60	0.50	0.50	0.39	0.39	0.40	0.39
dig-TSAA	0.87	0.87	0.78	0.78	0.65	0.65	0.63	0.63
dig-Threonine	0.77	0.77	0.66	0.66	0.62	0.62	0.53	0.53
Calcium	0.95	0.95	0.87	0.87	0.80	0.80	0.75	0.75
Available Phosphorus	0.45	0.45	0.42	0.42	0.38	0.38	0.36	0.36
Total Phosphorus	0.57	0.57	0.57	0.57	0.52	0.53	0.49	0.49
Sodium	0.20	0.20	0.20	0.20	0.18	0.20	0.18	0.18
Apparent Metabolizable	3102	2970	3124	2992	3168	3036	1450	1390
Analyzed Nutrient Content[5]								
Crude Protein	22.20	22.70	21.70	20.60	17.80	18.60	16.60	16.80
Crude Fat	4.93	3.49	5.30	2.90	6.31	4.55	6.25	4.24
Total Phosphorous	0.66	0.65	0.67	0.58	0.49	0.58	0.57	0.64
Acid Detergent Fiber	4.20	4.30	3.70	3.10	4.10	4.00	3.30	3.30

[1] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, and 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[2] Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate, and the premix contains less than 1% mineral oil.

[3] Active drug ingredient monensin sodium, 90 g/lb (90 g/ton inclusion; Elanco Animal Health, Indianapolis, IN), as an aid in the prevention of coccidiosis caused by *Eimeria necatrix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*.

[4] Optiphos; Huvepharma, St. Louis, MO.

[5]Analyzed enzyme recovery was 203 U/kg for starter, 197 U/kg for grower, and 191 U/kg for finisher, and 182 for withdrawal.

Table IV-3. Dietary formulations and calculated and analyzed nutrient content of the Positive Control (PC) and Negative Control (NC) fed to male market broilers in experiment 3.

	Starter (%)		Grower (%)		Finisher (%)	
Ingredient	**PC**	**NC**	**PC**	**NC**	**PC**	**NC**
Corn	56.03	60.48	62.33	64.07	67.47	66.83
Dehulled Soybean Meal (48%)	29.88	28.28	24.70	24.39	19.82	22.79
DL-Methionine (99%)	0.26	0.27	0.24	0.24	0.16	0.15
Dried Distillers Grain	5.00	5.00	5.00	5.00	5.00	5.00
Lysine HCL	0.23	0.27	0.21	0.22	0.19	0.09
Fat, A/V Blend	3.42	0.50	2.69	0.50	2.63	0.50
Limestone	1.02	1.03	0.83	1.59	0.75	0.74
Monocalcium Phosphate	0.39	0.39	0.18	0.17	0.11	0.09
Sodium Chloride	0.41	0.37	0.29	0.29	0.18	0.29
Vitamin Premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Trace Minerals[2]	0.05	0.05	0.05	0.05	0.05	0.05
Coban 90[3]	0.05	0.05	0.05	0.05	0.05	0.05
Phytase[4]	0.01	0.01	0.01	0.01	0.01	0.01
Calculated Nutrient Content						
Protein	22.50	22.15	20.50	20.50	18.50	19.78
dig-Lysine	1.18	1.18	1.04	1.04	0.90	0.90
dig-Methionine	0.57	0.57	0.53	0.53	0.43	0.43
dig-TSAA	0.85	0.85	0.80	0.80	0.67	0.68
dig-Threonine	0.70	0.69	0.64	0.64	0.56	0.61
Calcium	0.92	0.92	0.80	0.80	0.75	0.75
Available Phosphorus	0.45	0.45	0.40	0.40	0.38	0.38
Total Phosphorus	0.57	0.57	0.51	0.52	0.48	0.50
Sodium	0.20	0.20	0.20	0.20	0.20	0.20
Metabolizable Energy (kcal/kg)	3102	2970	3124	2992	3168	3036
Analyzed Nutrient Content[5]						
Crude Protein	21.90	20.50	19.60	19.90	17.10	18.60
Crude Fat	6.10	4.17	5.87	3.66	6.19	4.26
Total Phosphorous	0.66	0.57	0.60	0.63	0.56	0.55
Acid Detergent Fiber	3.20	2.70	3.30	3.00	2.70	2.80

[1] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, and 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[2] Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate, and the premix contains less than 1% mineral oil.

[3] Active drug ingredient monensin sodium, 90 g/lb (90 g/ton inclusion; Elanco Animal Health, Indianapolis, IN), as an aid in the prevention of coccidiosis caused by *Eimeria necatrix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*.

[4] Optiphos; Huvepharma, St. Louis, MO.

[5]Analyzed enzyme recovery was 393 U/kg for starter, 299 U/kg for grower, and 222 U/kg for finisher.

Statistical Analysis

All data were subjected to a one-way analysis of variance (ANOVA) using SPSS V 18.0 with significantly different means ($P \leq 0.05$) separated using Duncan's multiple range test. Percentage data (mortality and yield) were subjected to an arcsine transformation prior to statistical analysis.

Results

Experiment 1. During the starter period, the caloric reduction in the NC diet did not reduce BW compared to that of the PC diet (Table IV-4); however, the supplementation of NSPase increased ($P < 0.05$) BW by 13 g compared to that of the PC diet. On d 28, no differences in BW were observed between the PC and NC diets. Furthermore, the addition of NSPase in the reduced-energy diet did not improve BW when compared to that of the NC diet alone. At the conclusion of the trial, no differences in BW were observed among the treatments. With regard to FCR, the reduction in energy in the NC diet compared to that of the PC diet resulted in a 3 and 5 point increase ($P < 0.05$) during the starter and grower period, respectively (Table IV-4). The inclusion of NSPase in the energy-deficient diet improved ($P < 0.05$) starter FCR compared to that of the NC diet to levels similar to that of the PC. Following the starter period, NSPase supplementation in the NC did not significantly impact FCR throughout the remainder of the trial. The reduction in dietary energy in the NC diet increased ($P < 0.05$) cumulative d 1 to 28 FCR by 4 points compared to that of the PC diet. Although no differences in FCR were observed among the treatments during the finisher period, the reduction in

energy in the NC increased ($P < 0.05$) cumulative d 1 to 42 FCR by 4 points compared to that of the PC diet.

With regard to processing parameters, reducing the caloric content in the NC diet resulted in a 3.82% lighter ($P < 0.05$) individual broiler live weight compared to that of the PC diet (Table IV-4). Inclusion of NSPase in the reduced-energy diet increased the live weight of processed birds to levels that were comparable to those in the PC diet; however, improvements were not significantly different from those of the NC diet. A similar trend was observed in WOG weight, with the PC exhibiting a 3.83% increase ($P < 0.05$) in WOG weight compared to the reduced-energy diet; the addition of NSPase in the NC yielded intermediate results amongst dietary treatments. No differences in WOG yield were observed among the treatments. In both fat pad weight and yield, differences were observed between the PC and NC diets, with the PC yielding the highest ($P < 0.05$) fat pad weight and yield. The inclusion of NSPase did not impact fat pad weight or yield when compared to the NC diet.

Table IV-4. Average body weights, cumulative mortality-corrected feed conversion ratio (FCR) and processing parameters (± standard deviation SD) of male broilers fed low-energy diets with the inclusion of NSPase in experiment 1.

Phase	PC	NC[1]	NC+NSPase[2]	PSEM
Body Weight (kg)				
d14	$0.425^{b} \pm 0.012$	$0.435^{ab} \pm 0.009$	$0.438^{a} \pm 0.016$	0.002
d28	$1.512^{a} \pm 0.064$	$1.460^{ab} \pm 0.060$	$1.431^{b} \pm 0.063$	0.013
d42	2.658 ± 0.170	2.624 ± 0.107	2.611 ± 0.203	0.029
Mortality-Corrected Feed Conversion Ratio (feed:gain)				
Starter	$1.232^{b} \pm 0.018$	$1.268^{a} \pm 0.018$	$1.246^{b} \pm 0.034$	0.005
Grower	$1.459^{b} \pm 0.043$	$1.510^{a} \pm 0.051$	$1.515^{a} \pm 0.057$	0.011
Finisher	1.923 ± 0.077	1.941 ± 0.041	1.856 ± 0.174	0.021
d1-28	$1.399^{b} \pm 0.031$	$1.442^{a} \pm 0.034$	$1.462^{a} \pm 0.039$	0.008
d1-42	$1.625^{b} \pm 0.016$	$1.664^{a} \pm 0.015$	$1.634^{ab} \pm 0.055$	0.007
Processing Parameters				
Live Weight (g)	$2772^{a} \pm 249$	$2666^{b} \pm 179$	$2714^{ab} \pm 232$	16
WOG Weight (g)	$2115^{a} \pm 177$	$2037^{b} \pm 133$	$2068^{ab} \pm 173$	12
WOG Yield (%)	76.4 ± 1.5	76.4 ± 1.7	76.2 ± 1.7	0.1
Fat Pad Weight (g)	$36.7^{a} \pm 11.8$	$32.2^{b} \pm 9.5$	$29.8^{b} \pm 9.6$	0.8
Fat Pad Yield (%)	$1.8^{a} \pm 0.6$	$1.6^{b} \pm 0.4$	$1.4^{b} \pm 0.4$	0.1

[a-b] Treatment means within a row with different superscripts differ significantly at P≤0.05

[1] Energy level reduced by 132 kcal/kg compared to the PC.

[2] Inclusion of 113.5 g/ton (2,700 U/g of xylanase from *Aspergillus niger* and *Trichoderma reesei*; also contains ß glucanase and *α*-galactosidase) of NSPase (Enspira; United Animal Health, Sheridan, IN).

Experiment 2. The 132 kcal/kg ME reduction in the NC diet negatively impacted BW compared to that in the PC throughout the trial (Table IV-5). During the starter phase, the inclusion of NSPase increased ($P < 0.05$) BW by 75 g compared to that of the NC diet. On d 28, the reduction in energy in the NC diet reduced BW by 340 g compared to that of the PC diet. The supplementation of NSPase recovered 190 g of d 28 BW ($P < 0.05$) compared to that of the NC. Similar trends were observed on d 35 and 42, with the inclusion of NSPase increasing ($P < 0.05$) BW by 300 and 363 g, respectively, compared to that of the NC diet. During the starter, grower and finisher phases of the

experiment, differences in FCR were observed between the PC and NC diets, with the reduced-energy diet yielding the highest ($P < 0.05$) observed FCR among dietary treatments. During the starter phase, the inclusion of NSPase did not impact FCR compared to that of the NC diet. However, NSPase addition during the grower phase reduced FCR to levels similar to that of the PC-fed broilers. In the finisher phase, the inclusion of NSPase in the reduced-energy diet improved ($P < 0.05$) FCR by 13 points compared to that of the NC diet. Furthermore, birds fed diets containing NSPase exhibited an FCR similar to that of the PC during the finisher period. With regard to cumulative FCR, significant differences were observed through d 28, d 35, and d 42 between the PC and NC fed broilers, with the reduction in energy yielding the highest ($P < 0.05$) observed cumulative FCR at each evaluated cumulative time point. Regarding cumulative FCR through d 28, the supplementation of NSPase reduced FCR to levels comparable to those of the PC; however, FCR did not differ from the NC diet. Cumulative FCR through d 35 and 42 was reduced ($P < 0.05$) with the supplementation of NSPase in the NC diet while achieving levels similar to those in the PC diet.

With respect to processing, the negative impact observed on BW correlated with a reduction in carcass weight and yield. Higher energy levels in the PC increased ($P < 0.05$) fat pad weight and yield compared to those in the NC diet. The inclusion of NSPase in the reduced-energy diet increased ($P < 0.05$) both WOG weight and yield compared to those in the NC diet (Table IV-5), with WOG yields being similar to those in the PC. Supplementation with NSPase did not impact fat pad weight or yield compared to those in the energy-deficient diet.

Table IV-5. Average body weights and cumulative mortality-corrected feed conversion ratio (FCR) (± standard deviation SD) of male broilers fed low-energy diets with the inclusion of NSPase in experiment 2.

Phase	PC	NC[1]	NC+NSPase[2]	PSEM
Body Weight (kg)				
d14	$0.410^{a} \pm 0.013$	$0.310^{c} \pm 0.010$	$0.385^{b} \pm 0.018$	0.008
d28	$1.490^{a} \pm 0.055$	$1.150^{c} \pm 0.046$	$1.340^{b} \pm 0.042$	0.027
d35	$2.208^{a} \pm 0.043$	$1.675^{c} \pm 0.038$	$1.975^{b} \pm 0.100$	0.042
d42	$2.902^{a} \pm 0.042$	$2.339^{c} \pm 0.076$	$2.702^{b} \pm 0.102$	0.454
Mortality-Corrected Feed Conversion Ratio (feed:gain)				
Starter	$1.219^{b} \pm 0.050$	$1.300^{a} \pm 0.048$	$1.273^{a} \pm 0.030$	0.001
Grower	$1.477^{b} \pm 0.113$	$1.671^{a} \pm 0.215$	$1.543^{ab} \pm 0.088$	0.030
Finisher	$1.877^{b} \pm 0.102$	$2.000^{a} \pm 0.137$	$1.872^{b} \pm 0.147$	0.025
Withdrawal	$1.939^{a} \pm 0.095$	$1.870^{ab} \pm 0.113$	$1.835^{b} \pm 0.081$	0.019
d1-28	$1.407^{b} \pm 0.079$	$1.563^{a} \pm 0.143$	$1.475^{ab} \pm 0.049$	0.021
d1-35	$1.555^{b} \pm 0.045$	$1.694^{a} \pm 0.078$	$1.598^{b} \pm 0.035$	0.015
d1-42	$1.645^{b} \pm 0.043$	$1.738^{a} \pm 0.073$	$1.657^{b} \pm 0.031$	0.012
Processing Parameters				
Live Weight (g)	$2923^{a} \pm 199$	$2564^{c} \pm 224$	$2762^{b} \pm 212$	19
WOG Weight (g)	$2176^{a} \pm 162$	$1853^{c} \pm 302$	$2058^{b} \pm 170$	15
WOG Yield (%)	$74.40^{a} \pm 2.30$	$72.12^{b} \pm 9.72$	$74.50^{a} \pm 1.70$	0.2
Fat Pad Weight (g)	$28.73^{a} \pm 6.80$	$21.30^{b} \pm 8.01$	$23.08^{b} \pm 7.19$	0.6
Fat Pad Yield (%)	$1.32^{a} \pm 0.30$	$1.14^{b} \pm 0.34$	$1.11^{b} \pm 0.30$	0.1

[a-c] Means within columns with different superscripts differ significantly at $P \leq 0.05$

[1]Energy level reduced by 132 kcal/kg compared to the PC.

[2]Inclusion of 113.5 g/ton of NSPase (2,700 U/g of xylanase from *Aspergillus niger* and *Trichoderma reesei*; also contains ß glucanase and α-galactosidase) of NSPase (Enspira; United Animal Health, Sheridan, IN).

Experiment 3. The reduction in energy in the NC diet decreased ($P < 0.05$) BW at all evaluated time points (d 14, 28, and 42) compared to that of the PC (Table IV-6). On d 14, the inclusion of NSPase in the reduced-energy diet elevated ($P < 0.05$) BW compared to that of the NC diet to levels that were similar to those of the PC. The inclusion of NSPase increased ($P < 0.05$) BW by 84 g compared to that of the NC, with a numerical increase of 13 g being observed beyond that of the PC. NSPase

supplementation improved ($P < 0.05$) BW compared to that of the NC by 13 and 7.2% on d 28 and 42, respectively, to levels that were similar to those in the PC diet. During the starter phase, an increase ($P < 0.05$) in mortality-corrected FCR was observed, with the removal of energy in the NC diet increasing FCR by 10 points compared to the PC diet. The supplementation of NSPase in the low-energy diet reduced ($P < 0.05$) FCR to levels comparable to those in the PC. With regard to the grower phase, no differences were observed between the PC and NC diets. Through d 28, differences in cumulative FCR (d 1 to 28) were observed between the PC and the NC diets, with the PC yielding the lowest ($P < 0.05$) observed FCR. NSPase inclusion in the low-energy diet did not improve cumulative FCR (d 1 to 28) compared to that of the NC diet. During the finisher period and cumulative d 1 to 42, no differences in FCR were observed among treatments.

Table IV-6. Average body weights and cumulative mortality-corrected feed conversion ratio (FCR) (± standard deviation SD) of male broilers fed low-energy diets with the inclusion of NSPase in experiment 3.

Phase	PC	NC[1]	NC+NSPase[2]	PSEM
Body Weight (kg)				
d14	$0.436^{a} \pm 0.017$	$0.365^{b} \pm 0.022$	$0.449^{a} \pm 0.012$	0.008
d28	$1.448^{a} \pm 0.066$	$1.229^{b} \pm 0.062$	$1.412^{a} \pm 0.048$	0.021
d42	$2.764^{a} \pm 0.130$	$2.519^{b} \pm 0.140$	$2.713^{a} \pm 0.232$	0.036
Mortality-Corrected Feed Conversion Ratio (feed:gain)				
Starter	$1.227^{b} \pm 0.034$	$1.325^{a} \pm 0.086$	$1.211^{b} \pm 0.018$	0.013
Grower	$1.448^{b} \pm 0.025$	$1.462^{b} \pm 0.033$	$1.520^{a} \pm 0.045$	0.009
Finisher	1.926 ± 0.096	1.895 ± 0.094	1.923 ± 0.110	0.018
d1-28	$1.384^{b} \pm 0.021$	$1.423^{a} \pm 0.027$	$1.426^{a} \pm 0.030$	0.006
d1-42	1.642 ± 0.031	1.664 ± 0.036	1.662 ± 0.041	0.007

[a-b] Data in columns with different superscripts differ significantly at $P \leq 0.05$.

[1]Energy level reduced by 132 kcal/kg compared to the PC.

[2]Inclusion of 113.5 g/ton of NSPase (2,700 U/g of xylanase from *Aspergillus niger* and *Trichoderma reesei*; also contains ß glucanase and α-galactosidase) of NSPase (Enspira; United Animal Health, Sheridan, IN).

Discussion

These data indicate that the inclusion of exogenous enzymes in corn-soy diets containing 5 to 10% DDGS enhances the utilization of nutrients while improving both performance and FCR of male broilers. The inclusion of NSPase in the diet allows the manufacturer to reduce the caloric value through fat substitution with cereal grains such as corn or through dilution with high-fiber ingredients such as DDGS. In all three experiments, a 132 kcal/kg ME reduction in the NC reduced performance parameters when compared to the PC. In a study conducted by Coppedge et al. (2012), a 133 kcal/kg ME reduction impacted performance parameters, including a 20 g decrease in d 26 BW and an increase in FCR. The addition of a cocktail NSPase in the diet resulted in a 6

point improvement in starter and cumulative d 1 to 26 FCR. Similar results in broiler performance have been reported with the inclusion of carbohydrases in diets (Coppedge et al., 2012; Gracia et al., 2003; O'Neill et al., 2012b). It is widely accepted that the inclusion of exogenous enzymes in corn-soy diets has the potential to increase the overall feeding value of the diet while also improving bird performance.

Non-starch polysaccharides (NSP) have been shown to encapsulate vital nutrients such as starch and amino acids, resulting in a negative impact on broiler performance (Choct, 2006; Slominski, 2011). The use of exogenous enzymes in corn-soy diets mitigates these negative effects by hydrolyzing indigestible bonds, releasing these nutrients for utilization. Furthermore, the mode of action involving NSPase enzymes includes the degradation of viscous NSP as well as the liberation of encapsulated nutrients bound within the cell walls of various plant-based feedstuffs. The ability to release these inaccessible nutrients via NSPase supplementation allows greater digestion and absorption of protein, starch, and energy, resulting in greater BW gain and improved feed efficiency (Ravindran, 2013; Slominski, 2011). While a significant BW response was not observed between the PC and NC during experiment 1, it can be hypothesized that the removal of energy from the NC diet increased FC, resulting in an FCR response instead of a BW effect. The addition of NSPase in the reduced-energy diet yielded a 13 g increase ($P < 0.05$) in BW compared to that of the PC for the starter phase. Following the starter period, inclusion of cocktail NSPase in the NC did not impact BW throughout the trial. Similar results were observed by Yu and Chung (2004), in which improvements in performance parameters were not observed when evaluating the efficacy of a cocktail

carbohydrase supplemented in a reduced-energy (-3%) corn-soybean meal diet. The reduction in energy in the NC diet increased ($P < 0.05$) FCR compared to the PC during the starter and grower phases and cumulatively through d 28 and d 42. It can be assumed that the FCR response was attributed to an increase in FC due to the caloric reduction in the NC. Leeson et al. (1996) demonstrated that FC increased linearly with decreasing dietary energy levels. Furthermore, Hidalgo et al. (2004) noted that increasing dietary AME through fat supplementation improved FCR by reducing broiler FC. Avila et al. (2012) observed similar results with an energy reduction of 120 kcal/kg in the NC diet increasing both FC and FCR compared to the PC in 46 day old broilers. In the current trial, the supplementation of NSPase in the low-energy diet improved FCR to levels comparable to that of the PC during the starter period and cumulatively through d 42. Klein et al. (2015) observed similar results in two subsequent experiments when evaluating an NSPase in combination with a ß-mannanase in low-energy (-132 kcal/kg) diets. The addition of both NSPase and ß-mannanase improved starter (experiment 1) and finisher (experiment 2) FCR to levels equivalent to that of the PC diet.

The supplementation of cocktail carbohydrases in the diet has the ability to target and act on multiple substrates releasing nutrients within the specified substrates for utilization. In experiment 2 of the current study, the reduction in energy in the NC diet yielded a consistent decrease in BW throughout the trial compared to that of the PC diet. The inclusion of cocktail NSPase in the reduced-energy diet increased ($P < 0.05$) BW compared to that of the NC diet. These results were consistent with findings reported by Cowieson and Adeola (2005), in which a 28 d trial was conducted to evaluate the effects

of a multi-carbohydrase and phytase inclusion in reduced-energy (-180 kcal/kg) diets. Cowieson and Adeola (2005) reported that the inclusion of both enzymes at levels of 100 mg/kg yielded a 14% increase in BW compared to that of the NC diet. Additionally, Wu et al. (2004b) observed similar improvements in broiler performance with the inclusion of a carbohydrase in wheat-soy diets. With respects to FCR, a consistent increase was observed in the NC compared to the PC from the starter to the finisher phase. In the finisher phase (d 28 to 35), inclusion of NSPase in the reduced-energy diet improved FCR to levels similar to those in the PC diet. Similar results were observed by Cowieson et al. (2010), in which a 7 point decrease in FCR was achieved in reduced-energy diets (-110 kcal/kg) through xylanase and glucanase supplementation at levels of 100 and 150 g/ton, respectively, through d 42. O'Neill et al. (2012b) noted that a reduction of 100 kcal/kg of energy in NC diets increased ($P < 0.05$) FCR when compared to the PC diet. The supplementation of a xylanase product at levels of 16,000 BXU/kg in the reduced-energy diet improved ($P < 0.05$) (6 points) FCR at the conclusion of d 35.

In experiment 3, a decrease ($P < 0.05$) in BW was observed in the reduced-energy diet throughout the trial compared to that of the PC diet. Supplementing NSPase in the low-energy diet improved ($P < 0.05$) average BW by 14% compared to that of the NC diet alone. Cowieson et al. (2010) also observed an improvement in broiler performance when administering a carbohydrase in an energy-reduced (-150 kcal/kg) diet. Results observed by Olukosi et al. (2007) indicated that the inclusion of a multi-carbohydrase (XAP; 650 U xylanase, 1650 U amylase, and 4000 U protease) in a 28 d

trial yielded heavier BW when administered in a reduced-energy diet (-115 kcal/kg). Additionally, Francesch and Geraert (2009) reported that the multi-enzyme supplementation of phytase and carbohydrase in reduced-energy diets improved broiler BW through d 21. With regard to FCR, a reduction ($P < 0.05$) was observed between the PC and NC diets for the starter phase. Cocktail NSPase supplementation in the reduced-energy diet improved ($P < 0.05$) FCR to levels similar to the PC while yielding the lowest FCR for that period. Olukosi et al. (2007) also observed an improvement ($P < 0.05$) in FCR in low-energy diets supplemented with cocktail carbohydrase on d 21. Following the starter phase, no further improvements were observed when comparing the reduced-energy diet with NSPase inclusion to the NC diet alone. Coppedge et al. (2012) observed similar results with NSPase inclusion improving FCR compared to that of the NC diet in the starter phase while failing to influence FCR in the grower and finisher periods. When supplementing exogenous enzymes such as NSPase in the diet, bird responses to enzyme addition are not entirely predictable as seen in the series of these three experiments. In each experiment, a response was observed; however, the response varied from a BW response only to an FCR response only. Factors that impact these variations in response could include enzyme source and dose, dietary ingredients and factors, and bird characteristics including age and sex (Ravindran, 2013). Each of these three experiments illustrated a positive benefit with exogenous enzyme inclusion with either an impact on FCR or BW being more apparent. Sources of the variation in the response associated with these three trials may be ingredient variation, an environmental effect, or chick quality. Each trial was conducted during different

seasonal periods of the year in which both temperature and humidity may have impacted the bird's behavior and consumption and the subsequent responses observed. Although varying responses were observed with enzyme supplementation, the removal of energy (-132 kcal/kg) in the NC diet throughout all experiments seemed to successfully elicit a consistent response between the NC and PC with differences in performance parameters being significant in each experiment. When 4% energy was removed in the NC, broiler performance was impacted, with reductions in BW and increases in FCR being observed. Similar differences between the PC and NC were reported by Klein et al. (2015) and Williams et al. (2014b), in which a 132 kcal/kg reduction in energy-reduced BW and increased FCR were reported, demonstrating that a 4% reduction in dietary energy is sufficient for adequate separation between the PC and NC diets.

While the incorporation of exogenous enzymes in broiler diets have shown to increase broiler performance, studies have also indicated the ability of enzyme supplementation to improve processing yields as well (Coppedge et al., 2012; Gehring et al., 2011; Williams et al., 2014b). In experiment 1, the reduction in energy in the NC diet decreased processing parameters, including live weight, carcass weight, and fat pad weight and yield compared to the PC diet. Williams et al. (2014b) observed similar results, with the reduction in energy (-132 kcal/kg) in the diet decreasing both fat pad weight and yield. The inclusion of NSPase in the low-energy diet yielded live weights and carcass weights similar to those of the PC. In experiment 2, a reduction ($P < 0.05$) in the NC was observed in all processing parameters (live weight, WOG weight and yield, fat pad weight and yield) when compared to the PC diet. The inclusion of NSPase in the

reduced-energy diet improved ($P < 0.05$) live weight, WOG weight and yield compared to those in the NC diet. Similar results were observed by Coppedge et al. (2012), in which the inclusion of NSPase in reduced-energy diets improved both live BW and carcass weights. In the current studies, the supplementation of a cocktail NSPase in reduced-energy corn-soybean meal diets with DDGS inclusion improved broiler performance and processing parameters. Observations conclude that the administration of NSPase in reduced-energy diets can compensate for caloric reduction while improving broiler growth and feed conversion. The results of this study indicate that through NSPase supplementation, producers can successfully incorporate by-product ingredients such as DDGS while reducing the caloric value of the diet without negatively impacting broiler performance. The addition of NSPase in low-energy broiler diets has the ability to target various substrates, degrading essential linkages crucial for enhanced nutrient digestion, which can ultimately improve broiler performance parameters and reduce production costs.

CHAPTER V

EFFECT OF INCREASING PHYTASE INCLUSION LEVELS ON BROILER, PERFORMANCE, NUTRIENT DIGESTIBILITY, AND BONE MINERALIZATION IN LOW-PHOSPHORUS DIETS.

Introduction

Feeds formulated for monogastric animals such as poultry and swine are primarily composed of plant-based feedstuffs, with a majority of the constituents deriving from the seeds of plants. Approximately 60 to 80% of phosphorus (P) in plants is stored in the form of phytate (*myoinositol 1,2,3,4,5,6- hexakis dihydrogen phosphate*) with corn and soybean meal diets typically containing 8.0 to 9.0 g of phytate per kilogram of feed (Cabahug et al., 1999). As a result of inadequate endogenous phytase activity in poultry, phytate utilization and digestion is quite poor, warranting the need for inorganic phosphate supplementation to fulfill the P requirement (Cowieson et al., 2006a; Wendt and Rodehutscord, 2004). The inclusion of feed phosphates in poultry diets represent a considerable cost factor with P being the third most expensive ingredient following energy and protein (Boling et al., 2000). Furthermore, the antinutritive properties associated with phytate can lead to the formation of insoluble complexes through mineral chelation and nutrient binding as well as increases in endogenous losses, thus impeding nutrient digestion and adversely affecting bird performance (Cowieson et al., 2004; Selle et al., 2000; Woyengo and Nyachoti, 2013).

It is well known that phytase fed at conventional levels (≤ 500 FTU/kg) ameliorates the anti-nutritive properties of phytate through dephosphorylation resulting in improvements in the digestibility of P as well as other nutrients bound to the inositol ring (Powell et al., 2011; Ravindran et al., 1999; Ravindran et al., 2008; Selle et al., 2000). Additionally, lower doses of phytase have been supplemented in an effort to better utilize plant P in the diet, increase animal performance, reduce inorganic P inclusion, and limit environmental P pollution (Bedford, 2000; Kumar et al., 2015; Lenis and Jongbloed, 1999; Selle and Ravindran, 2007). While this practice has been considered common, super-dosing (> 1500 FTU/kg) phytase has become a growing opportunity for poultry producers due to rising ingredient costs and lower enzyme prices. Super-dosing phytase has shown to further increase phytate degradation, improve nutrient availability, and perhaps generate myo-inositol with vitamin-like/lipotropic effects (Cowieson et al., 2011; Pirgozliev et al., 2011; Shirley and Edwards, 2003). Pieniazek et al. (2017) reported that higher levels of phytase (2,000 U/kg) further improved amino acid digestibility with a 9.1% increase in feed intake resulting in a 142 g heavier bird weight compared to conventional doses (500 FTU/kg). Additionally, Cowieson et al. (2006b) observed greater improvements in nutrient utilization, feed consumption (FC), and body weight (BW) as phytase supplementation was increased in logarithmic doses from 150 to 24,000 FTU/kg in low-P diets. Although not fully understood, these "extra-phosphoric effects" translate to improvements in BW gain, FC, and FCR beyond that of traditional doses (Augspurger and Baker, 2004; Cowieson et al., 2006b; Kies et al., 2006; Manobhavan et al., 2015; Pirgozliev et al., 2011; Pirgozliev et al., 2008; Shirley and Edwards, 2003). The purpose

of the current study was to evaluate the effects of increasing levels of phytase on bone mineralization, nutrient digestibility, and broiler performance when fed in low-P diets.

Materials and Methods

Experimental Design

The microbial phytase (Natuphos® E[1]) contained 10,000 phytase units (FTU)/g of phytase activity. One FTU is defined as the quantity of enzyme required to release 1 µmol of inorganic P/min from 0.00015 mol/L sodium phytate at pH 5.5 at 37 C.

A total of 2,800 Cobb 500 male broiler chicks were obtained from a commercial hatchery and randomized according to weight and placed in 1.672 m^2 floor pens on litter previously used by 3 flocks. Chicks were randomly assigned to 8 dietary treatments, each with 12 replicates with 35 broilers per replicate. Birds were raised under conventional conditions with age appropriate lighting, temperature, and ventilation. Diets were fed ad-libitum throughout the study with the starter (d 1 to 14) being fed as a crumble diet and grower (d 15 to 28) being fed as a pellet. Body weight (BW), mortality adjusted feed conversion (FCR), feed consumption (FC) and mortality were determined on d 14 and 28.

Experimental Diets

A study was conducted to determine the impact of increasing levels of phytase inclusion in low-P diets. The experimental design consisted of eight corn-soybean meal-based diets with the positive control (PC) being a standard corn-soybean meal diet with a calculated non-phytate phosphorus (nPP) level of 0.43 and 0.39% for the starter and

[1] Natuphos® E- BASF Inc. Florham Park, NJ

grower, respectively. The negative control (NC) included a reduction in nPP to 0.25 and 0.23% for the starter and grower, respectively, compared to the PC. Dietary treatments were created by dividing the NC diet into six additional treatments consisting of phytase at levels of 250, 500, 750, 1,000, 2,000, and 3,000 FTU/kg. All phytase levels were fed throughout both the starter and grower with the exception of the 3,000 FTU/kg treatment, which utilized a step-down program consisting of 3,000 FTU/kg in the starter period decreasing to 1,000 FTU/kg in the grower phase. All diets were formulated to be isonitrogenous and isocaloric with phytase being supplemented in the experimental diets during mixing and prior to pelleting. Mash feed was subjected to a 12 second conditioning time and a pelleting temperature of 70°C. Titanium Dioxide (TiO) was added to all diets during both the starter and grower period at an inclusion rate of 0.4% and used as an indigestible marker to determine nutrient digestibility. Crude protein was determined using AOAC by combustion (AOAC 990.03), total P determined by wet ash ICP (AOAC 985.01M), acid detergent fiber determined using an ANKOM digestion unit (AOAC 973.18), and an ether extraction to determine crude fat (AOAC 920.39). Both mash and pelleted samples were collected in duplicates during the feed manufacturing period and analyzed for nutrient content and phytase recovery (Table V-2).

Table V-1. Dietary composition and calculated nutrient content of low-phosphorus diets with increasing phytase inclusion levels.

	Starter		Grower	
Ingredient	**PC**	**NC**	**PC**	**NC**
Corn	57.14	58.49	60.99	62.40
Dehulled Soybean Meal (48%)	35.59	35.35	31.69	31.20
DL-Methionine (99%)	0.27	0.26	0.25	0.25
Lysine HCL	0.13	0.14	0.09	0.10
L-Threonine	0.06	0.06	0.01	0.01
Soy Oil	2.79	2.32	3.22	2.79
Limestone	1.37	1.57	1.25	1.50
Monocalcium Phosphate	1.46	0.61	1.29	0.53
Salt	0.46	0.46	0.46	0.44
Sodium Bicarbonate	0.00	0.00	0.01	0.03
Trace Minerals[1,2]	0.05	0.05	0.05	0.05
Vitamins[3]	0.25	0.25	0.25	0.25
Salinomycin	0.05	0.05	0.05	0.05
Titanium Dioxide	0.40	0.40	0.40	0.40
Calculated Nutrient Concentration				
Protein	22.44	22.44	20.79	20.69
Digestible Lysine	1.18	1.18	1.05	1.05
Digestible Methionine	0.57	0.57	0.54	0.54
Digestible TSAA	0.87	0.87	0.82	0.82
Digestible Threonine	0.79	0.79	0.68	0.68
Calcium	0.86	0.80	0.78	0.75
Available Phosphorus	0.43	0.25	0.39	0.23
Total Phosphorus	0.69	0.51	0.64	0.48
Sodium	0.20	0.20	0.20	0.20
Metabolizable Energy (kcal/kg.)	3036	3036	3102	3102

[1]Trace mineral premix added in the starter yields 180 mg of manganese, 108 mg of total zinc, 5.1 mg of copper, 3.51 mg of iodine, 0.3 mg of total selenium, and 0.013 g of *bacillus subtilis*.

[2]Trace mineral premix added in the grower yields 150 mg of manganese, 90 mg of total zinc, 4.25 mg of copper, 2.92 mg of iodine, 0.25 mg of total selenium, and 0.011 g of *bacillus subtilis*.

[3] Vitamin premix added at this rate yields 7700 IU vitamin A, 5500 ICU vitamin D_3, 55 IU vitamin E, 1.5 mg vitamin K-3, 0.01 mg B_{12}, 6.6 mg riboflavin, 38.5 mg niacin, 9.9 mg d-pantothenic acid, 0.88 mg folic acid, 2.75 mg pyridoxine, 1.54 mg thiamine, 0.08 mg biotin per kg diet

Chemical Analysis

On d 14 and 28, 8 and 6 birds respectively, for the starter and grower phase were euthanized via carbon dioxide asphyxiation and tibias and ileal digesta were sampled. Right tibias were collected, adhering tissue removed, and dried for 24 h at 100°C. Tibias

were fat extracted via Soxhlet extraction using 100% ethyl ether according to modified methods of Watson et al. (2006). Following extraction, tibias were dried at 100°C for 24 h and ashed for 24 h at 600°C using a muffle furnace. Tibias were analyzed for ash weight, P, and Ca. Tibia P and Ca content were analyzed using AOAC method 2011.14.

Digesta was obtained from the terminal ileum midway between Meckel's diverticulum to 2 cm anterior to the ileocecal junction, pooled per replicate, and frozen at -5°C. Samples were then freeze dried via lypholizer (FreezeZone® Freeze Dry Systems) and ground for nutrient analysis and titanium concentration. Titanium concentration was determined using a modified protocol outlined by Short et al. (1996). Using this method, dried ileal digesta were weighed with 0.5 g of each sample being ashed and titrated with 10 mL of sulfuric acid (7.4 M). Samples were then boiled at 200°C for 2 h until fully dissolved, titrated with 20 mL of 30% H_20_2 and brought to 100 mL using distilled water. Absorption analysis was conducted using a Thermo Fisher Scientific Genesys 10S UV-Vis (Model 10S UV-Vis) spectrophotometer (Thermo Fisher Scientific, Waltham, MA) at 410 nm. Gross energy of feed and ileal digesta were determined using a Parr 6400 bomb calorimeter (Parr Instrument Company, Moline, IL) and ileal digestible energy (IDE) was calculated as:

$$IDE = Gross\ Heat_{Diet} - (Gross\ Heat_{ileum}\ x\ (\frac{TiO_{2\ Diet}}{TiO_{2\ ileum}})$$

Apparent ileal digestibility (AID) of Ca and P was determined using AOAC method 2011.14. Total amino acid profile was determined at the Experiment Station Chemical Laboratories, University of Missouri, using official method AOAC 982.30 E (a,b,c). All amino acids were analyzed except methionine, cysteine, and tryptophan

following 22-h acid hydrolysis (method 982.30E, step a;). Methionine and cysteine were analyzed after oxidation hydrolysis with performic acid (method 982.30E, step b;). Tryptophan was analyzed after alkali oxidation using 4.2 M NaOH and boiling at 110°C for 24 h (method 982.30E, step c;). The amino acid concentration of samples was quantified using HPLC. To determine AID of P and Ca, values were calculated using the following equation:

$$AID = \frac{[\left(\frac{nutrient}{TiO_2}\right) diet - \left(\frac{nutrient}{TiO_2}\right) ileum]}{(nutrient/TiO_2)diet}$$

Statistical Analysis

All data were analyzed via a one-way analysis of variance (ANOVA) using the General Linear Model procedure using SPSS V 22.0 (2015). Treatment means were deemed significantly different at $P \leq 0.05$ and separated using Duncan's multiple range test. The effect of phytase supplementation levels was determined using orthogonal polynomial contrast test for linear and quadratic effect.

Results

Diet Analysis

Analyzed nutrient values and phytase recovery values are shown in Table V-2. Analysis of phytase activity in experimental diets indicated values were within an acceptable range and represented regular and super-doses.

Table V-2. Analyzed nutrient content of low-phosphorus diets with increasing phytase inclusion levels.

	Starter		Grower	
Treatment	**PC**	**NC**	**PC**	**NC**
Moisture (%)	12.44	12.21	12.24	11.71
Dry matter (%)	87.56	87.79	87.76	88.29
Protein (crude) (%)	21.80	22.40	18.9	20.20
Fat (crude) (%)	5.12	4.88	5.87	5.36
Fiber (acid detergent) (%)	3.60	3.10	2.60	2.00
Ash (%)	5.41	5.00	4.79	4.75
Sulfur (total) (%)	0.27	0.27	0.26	0.24
Phosphorus (total) (%)	0.75	0.57	0.71	0.54
Potassium (total) (%)	1.06	1.02	0.94	0.98
Magnesium (total) (%)	0.17	0.17	0.16	0.16
Calcium (total) (%)	1.00	1.06	1.03	0.92
Sodium (total) (%)	0.19	0.26	0.21	0.18
Iron (total) ppm	301	218	323	213
Manganese (total) ppm	85.20	145	101	88.30
Copper (total) ppm	16.30	18.20	17.90	21.80
Zinc (total) ppm	89.80	108	106	89.90

Phytase Recovery Analysis (FTU/kg)						
Target Activity	**250**	**500**	**750**	**1,000**	**2,000**	**3,000/1,000**
Starter (d 1-14)	315	420	781	1,165	1,859	2,832
Grower (d 14-28)	325	569	812	1,064	1,772	1,064

Nutrient Digestibility

The reduction of nPP in the NC diet decreased ($P < 0.05$) ileal digestibility of P by 17.61 and 10.39% on d 14 and 28, respectively, compared to the PC (Table V-3). Increasing doses of phytase increased (linear, $P < 0.001$; quadratic, $P < 0.001$) P digestibility on both d 14 and 28. Improvements in P digestibility ranged from 6.64 with 250 FTU/kg to 22.27% with 3,000/1,000 FTU/kg on d 14 with birds fed phytase doses

500 FTU/kg or greater yielding P digestibility similar to that of the PC. Similarly, on d 28, ileal digestibility of P ranged from 9.54 (250 FTU/kg) to 25.24% (2,000 FTU/kg) with birds fed phytase at 750 and 2,000 FTU/kg improving ($P < 0.001$) P digestibility beyond that of the PC. No differences in Ca digestibility were detected between the PC and NC on d 14. However, linear and quadratic effects were identified ($P < 0.05$) with phytase supplementation. Increasing phytase inclusion up to 1,000 FTU/kg did not affect Ca digestibility compared to the NC, however, higher doses (2,000 and 3,000/1,000 FTU/kg) reduced Ca digestibility to levels similar to that of the PC diet. Birds fed the NC had 14.51% higher ($P < 0.05$) Ca digestibility compared to birds fed the PC on d 28. Additionally, phytase inclusion, regardless of dose, did not influence Ca digestibility compared to the NC on d 28. Although no differences in IDE were detected on d 14, birds fed the NC diet exhibited a 337 kcal/kg reduction in IDE values compared to those fed the diet adequate in P on d 28. With phytase addition, linear and quadratic effects ($P < 0.05$) were observed as phytase activity increased IDE values to levels comparable to that of the PC. Inclusion of super-doses of phytase (2,000 and 3,000/1,000 FTU/kg) yielded the greatest improvement in d 28 IDE with an increase of 281 and 282 kcal/kg, respectively.

Table V-3. Tibia bone mineralization and ileal digestibility from broilers fed low-phosphorus diets with increasing phytase inclusion levels.

		Tibia Bone Mineralization (%)						Ileal Digestibility					
		Bone Ash		Calcium		Phosphorus		IDE		Calcium		Phosphorus	
TRT	**Treatment**	**d 14**	**d 28**	**d 14**	**d 28**	**d 14**	**d 28**	**d 14**	**d 28**	**d 14**	**d 28**	**d 14**	**d 28**
1	Positive Control (PC)	49.95^{b}	50.65^{a}	37.05^{a}	37.41^{a}	17.62^{bc}	17.81^{a}	3073	3148^{a}	55.42^{bc}	43.28^{b}	53.91^{ab}	54.43^{cde}
2	Negative Control (NC)	42.12^{g}	42.98^{d}	36.42^{c}	37.04^{b}	16.53^{f}	16.68^{e}	2883	2811^{b}	61.44^{ab}	57.79^{a}	36.30^{d}	44.04^{f}
3	NC+ 250 FTU/kg	45.81^{f}	46.42^{c}	36.70^{b}	37.51^{a}	17.07^{e}	17.35^{d}	2928	2971^{ab}	63.73^{a}	57.88^{a}	42.94^{cd}	53.58^{de}
4	NC+ 500 FTU/kg	46.68^{e}	48.23^{b}	36.82^{ab}	37.30^{ab}	17.30^{d}	17.48^{cd}	2918	2939^{ab}	62.62^{ab}	52.43^{ab}	48.83^{bc}	52.04^{e}
5	NC+ 750 FTU/kg	48.29^{d}	48.89^{b}	36.76^{ab}	37.56^{a}	17.52^{c}	17.58^{bc}	2886	2982^{ab}	60.42^{ab}	56.28^{a}	49.93^{bc}	62.69^{ab}
6	NC+ 1000 FTU/kg	48.88^{c}	48.21^{b}	36.92^{ab}	37.34^{ab}	17.53^{c}	17.58^{bc}	2820	3027^{ab}	59.37^{ab}	51.65^{ab}	46.00^{bc}	62.00^{abc}
7	NC+ 2000 FTU/kg	50.39^{ab}	50.41^{a}	36.89^{ab}	37.32^{ab}	17.71^{ab}	17.67^{ab}	2876	3092^{a}	49.18^{c}	56.18^{a}	51.57^{ab}	69.28^{a}
8	NC+ 3000/1000 FTU/kg	50.65^{a}	49.99^{a}	36.87^{ab}	37.57^{a}	17.78^{a}	17.73^{ab}	2937	3091^{a}	55.11^{bc}	48.51^{ab}	58.57^{a}	61.07^{bcd}
ANOVA													
	Pooled SEM	0.45	0.38	0.08	0.09	0.08	0.07	26	31	1.54	1.65	1.63	1.63
	Probability (P-value)	<0.001	<0.001	<0.001	0.030	<0.001	<0.001	0.06	0.023	<0.001	0.005	<0.001	<0.001
REGRESSION													
	Linear[1]	<0.001	<0.001	0.047	0.614	<0.001	<0.001	0.83	0.003	0.002	0.609	<0.001	<0.001
	Quadratic[2]	<0.001	<0.001	0.021	0.283	<0.001	<0.001	0.58	0.006	0.005	0.350	<0.001	<0.001

[1]Linear effects of supplementing increasing levels of phytase

[2]Quadratic effects of supplementing increasing levels of phytase

Tibia Mineralization

The reduction of nPP in the NC decreased ($P < 0.001$) tibia ash percent by 7.83 and 7.67%, respectively, compared to the PC on d 14 and 28 (Table V-3). Additionally, birds fed the NC exhibited the lowest ($P < 0.05$) Ca and P content amongst dietary treatments. Bone mineralization, as indicated by tibia ash percent, linearly and quadratically improved ($P < 0.001$) with increasing levels of supplemental phytase on d 14 and 28. Incorporating increasing doses of phytase improved (linear and quadratic, $P < 0.001$) tibia P throughout the duration of the trial. The addition of phytase regardless of dose, increased ($P < 0.001$) tibia ash percent and P content throughout the study when compared to the NC diet. On d 14, the inclusion of phytase at 2,000 FTU/kg yielded P and tibia ash percent values similar to that of the PC while the addition of the highest level of phytase (3,000/1,000 FTU/kg) increased P and ash percent beyond that of the PC. A similar trend was observed for tibia P and ash percent on d 28 with super-dosing levels of phytase (2,000 and 3,000/1,000 FTU/kg) increasing values to levels comparable to that of the PC. Calcium content was adversely affected ($P < 0.001$) when feeding the low P diet compared to the PC on d 14 and 28. Supplementing phytase linearly and quadratically ($P < 0.05$) increased tibia Ca content with doses of 500 FTU/kg and greater increasing Ca levels similar to that of the PC. On d 28, the inclusion of phytase at 250, 750, and 3,000/1,000 FTU/kg increased tibia Ca content beyond ($P < 0.05$) that of the NC to levels comparable to that of the PC diet.

Amino Acid Digestibility

Feeding birds low-nPP diets decreased ($P < 0.05$) in the digestibility of all amino acids on d 14 and 28 with a decrease of 9.23 and 9.46%, respectively, in total amino acid digestibility compared to birds fed diets adequate in nPP (Table V-4). Supplementing phytase, depending on dose, improved phenylalanine (750, 1,000, 2000, and 3,000/1,000 FTU/kg), methionine and leucine (2,000 and 3,000/1,000 FTU/kg), and arginine (3,000/1,000 FTU/kg) on d 14. Increasing phytase concentrations linearly and quadratically ($P < 0.05$) improved the digestibility of phenylalanine, methionine, and arginine with linear ($P < 0.05$) increases in valine, isoleucine, histidine, leucine, and total amino acid digestibility on d 14. On d 28, the addition of phytase, regardless of dose, improved ($P < 0.05$) the digestibility of all amino acids compared to the NC diet with an increase in total amino acid digestibility ranging from 5.05 (250 FTU/kg) to 7.65% (2,000 FTU/kg). Linear and quadratic ($P < 0.05$) increases in d 28 amino acid digestibility were observed for all amino acids evaluated at the conclusion of the study. Including phytase at 2,000 FTU/kg yielded the greatest improvement amongst phytase treatments for all amino acids evaluated on d 28.

Table V-4. Amino acid digestibility (%) from broilers fed low-phosphorus diets with increasing phytase (FTU/kg) inclusion levels.

Amino Acid	PC	NC	NC+ 250	NC+ 500	NC+ 750	NC+ 1000	NC+ 2000	NC+ 3000/1000	P-value	SEM	Linear[1]	Quadratic[2]
						d 14						
Phenylalanine	80.64^{ab}	76.83^{d}	77.73^{cd}	78.01^{cd}	79.08^{bc}	79.20^{bc}	80.92^{a}	80.90^{a}	<0.001	0.29	<0.001	<0.001
Valine	78.16^{a}	66.23^{b}	69.01^{b}	69.87^{b}	68.83^{b}	67.66^{b}	71.23^{b}	71.25^{b}	<0.001	0.68	0.049	0.132
Threonine	73.11^{a}	61.44^{b}	63.16^{b}	64.69^{b}	63.47^{b}	61.48^{b}	65.17^{b}	65.96^{b}	0.001	0.72	0.120	0.287
Tryptophan	83.91^{a}	77.05^{b}	77.98^{b}	78.92^{b}	78.00^{b}	77.82^{b}	79.77^{b}	79.98^{b}	0.005	0.48	0.075	0.202
Isoleucine	79.34^{a}	69.24^{b}	71.19^{b}	72.30^{b}	71.33^{b}	70.25^{b}	73.76^{b}	73.71^{b}	<0.001	0.60	0.033	0.098
Methionine	88.08^{a}	82.64^{c}	84.27^{bc}	84.75^{bc}	84.44^{bc}	83.52^{bc}	86.24^{ab}	86.19^{ab}	0.002	0.40	0.015	0.047
Histidine	82.35^{a}	74.04^{b}	75.25^{b}	76.55^{b}	75.53^{b}	74.67^{b}	77.96^{b}	77.76^{b}	0.001	0.50	0.020	0.064
Arginine	87.78^{a}	79.80^{c}	80.61^{c}	82.16^{bc}	82.53^{bc}	81.60^{bc}	82.55^{bc}	84.39^{b}	<0.001	0.48	0.009	0.031
Lysine	82.02^{a}	71.89^{b}	74.56^{b}	75.27^{b}	74.69^{b}	73.30^{b}	76.24^{b}	76.49^{b}	<0.001	0.62	0.069	0.176
Leucine	79.71^{a}	70.43^{c}	72.41^{bc}	73.44^{bc}	72.71^{bc}	71.66^{bc}	75.28^{b}	74.99^{b}	0.001	0.57	0.020	0.060
Total	80.15^{a}	70.92^{b}	72.35^{b}	73.62^{b}	72.79^{b}	71.61^{b}	74.99^{b}	74.88^{b}	<0.001	0.55	0.031	0.093
						d 28						
Phenylalanine	83.15^{a}	73.11^{c}	78.69^{b}	79.65^{ab}	80.05^{ab}	80.04^{ab}	81.40^{ab}	79.82^{ab}	<0.001	0.57	0.002	0.001
Valine	80.89^{a}	69.45^{c}	75.37^{b}	76.44^{ab}	76.95^{ab}	76.76^{ab}	78.31^{ab}	76.32^{ab}	0.001	0.66	0.004	0.002
Threonine	75.86^{a}	63.39^{c}	69.50^{b}	70.02^{ab}	71.25^{ab}	71.08^{ab}	72.71^{ab}	70.58^{ab}	0.003	0.76	0.006	0.004
Tryptophan	85.91^{a}	70.87^{b}	80.36^{a}	81.74^{a}	83.07^{a}	82.52^{a}	84.37^{a}	82.29^{a}	0.008	1.02	0.013	0.006
Isoleucine	81.58^{a}	70.50^{c}	76.31^{b}	77.34^{ab}	77.91^{ab}	77.60^{ab}	79.34^{ab}	77.31^{ab}	<0.001	0.63	0.002	0.001
Methionine	91.08^{a}	84.32^{b}	88.05^{a}	89.20^{a}	88.84^{a}	88.94^{a}	89.78^{a}	88.34^{a}	0.001	0.41	0.005	0.002
Histidine	85.04^{a}	77.15^{b}	81.39^{a}	82.22^{a}	83.01^{a}	82.11^{a}	83.45^{a}	82.74^{a}	0.002	0.48	0.006	0.002
Arginine	90.18^{a}	84.82^{b}	88.29^{a}	89.32^{a}	88.56^{a}	88.88^{a}	89.76^{a}	89.01^{a}	0.005	0.37	0.009	0.005
Lysine	85.07^{a}	75.63^{b}	80.53^{a}	81.69^{a}	81.72^{a}	81.57^{a}	83.35^{a}	80.93^{a}	0.003	0.59	0.005	0.004
Leucine	83.15^{a}	72.78^{c}	78.61^{b}	79.65^{ab}	79.73^{ab}	80.01^{ab}	81.18^{ab}	79.72^{ab}	<0.001	0.58	0.002	0.001
Total	83.02^{a}	73.56^{c}	78.61^{b}	79.38^{ab}	80.04^{ab}	79.83^{ab}	81.21^{ab}	79.65^{ab}	0.001	0.55	0.003	0.001

[1]Linear effects of supplementing increasing levels of phytase

[2]Quadratic effects of supplementing increasing levels of phytase

Performance Parameters

Reducing nPP levels in the NC diet decreased ($P < 0.05$) FC by approximately 24% during the starter (27.9 g vs. 36.9 g) and 27% in the grower (91.1 g vs. 125.3 g) compared to birds fed the PC (Table V-5). This decrease in FC resulted in a reduction ($P < 0.05$) in BW by 93 and 536 g respectively, on d 14 and 28 when compared to the PC. Furthermore, birds fed the NC exhibited the highest FCR ($P < 0.05$) and mortality ($P < 0.05$) amongst treatments during both the grower phase and cumulatively (d 1 to 28). The inclusion of increasing levels of phytase linearly ($P < 0.001$) and quadratically ($P < 0.001$) increased FC and BW throughout the study. Increases in starter FC ranged from 4.6 (250 FTU/kg) to 10.8 g (3,000/1,000 FTU/kg) when adding phytase to the low-nPP diet. Greater FC with phytase inclusion resulted in a 56 (250 FTU/kg) to 79 g (3,000/1,000 FTU/kg) heavier d 14 BW compared to the NC. Doses of phytase at 750 and 1,000 FTU/kg produced BW similar to that of the PC while super-dosing (2,000 and 3,000/1,000 FTU/kg) further increased both FC and broiler BW beyond that of the PC diet. Although no differences in starter FCR were detected between the PC and NC, increasing phytase inclusion reduced (linear and quadratic, $P < 0.05$) FCR with the addition of phytase at 500, 2,000, and 3,000/1,000 FTU/kg decreasing ($P < 0.05$) starter FCR compared to both control diets. Similar trends in performance were observed on d 28 with phytase addition increasing ($P < 0.05$) grower and cumulative (d 1 to 28) FC regardless of activity level. Additionally, super-dosing phytase (2,000 and 3,000/1,000 FTU/kg) yielded responses in FC similar to that of the PC. Higher FC in phytase supplemented diets resulted in heavier BW on d 28 with 2,000 FTU/kg phytase yielding the heaviest BW amongst treatments.

The reduction of nPP in the NC diet increased ($P < 0.001$) grower and cumulative FCR compared to the PC with phytase supplementation improving (linear, $P < 0.05$; quadratic, $P < 0.001$) grower and cumulative (d 1 to 28) FCR compared to the NC regardless of concentration. Although treatment effects were not observed for starter mortality, birds fed the NC exhibited higher ($P < 0.05$) grower and cumulative (d 1 to 28) mortality compared to those fed the PC with phytase inclusion reducing mortality back to the PC diet. Furthermore, supplementing increasing doses of phytase resulted in linear and quadratic ($P < 0.05$) reductions in grower mortality and quadratic ($P < 0.05$) decreases in cumulative (d 1 to 28) mortality. Birds fed the "step-down" phytase program (3,000/1,000 FTU/kg) exhibited the highest FC, heaviest BW, and lowest FCR amongst phytase treatments during the starter period. However, reducing phytase activity to 1,000 FTU/kg in the "step-down" treatment during the grower phase resulted in a lower magnitude of improvement in performance at the conclusion of the trial compared to super-dose levels (2,000 FTU/kg) fed throughout the study.

Table V-5. Growth performance of male broilers fed low-phosphorus diets with increasing phytase inclusion levels.

		Body Weight		Feed Conversion Ratio			Feed Consumption			Mortality (%)		
TRT	**Treatment**	**d 14**	**d 28**	**Starter**	**Grower**	**d 1-28**	**Starte**	**Grower**	**d 1-28**	**Starte**	**Grower**	**d 1-28**
1	Positive Control (PC)	0.459^{c}	1.565^{ab}	1.257^{a}	1.588^{d}	1.478^{cd}	36.9^{b}	125.3^{a}	75.0^{a}	1.43	0.00^{b}	1.50^{b}
2	Negative Control (NC)	0.366^{f}	1.029^{f}	1.244^{ab}	1.973^{a}	1.676^{a}	27.9^{f}	91.1^{e}	54.1^{e}	4.50	2.30^{a}	6.75^{a}
3	NC+ 250 FTU/kg	0.422^{e}	1.280^{e}	1.220^{bc}	1.581^{d}	1.446^{e}	32.5^{e}	96.6^{d}	59.7^{d}	2.39	0.50^{b}	3.00^{b}
4	NC+ 500 FTU/kg	0.434^{d}	1.368^{d}	1.216^{c}	1.678^{b}	1.512^{b}	33.6^{d}	111.6^{c}	67.0^{c}	2.86	0.74^{b}	3.67^{b}
5	NC+ 750 FTU/kg	0.457^{c}	1.448^{c}	1.224^{bc}	1.658^{b}	1.503^{bc}	35.6^{c}	117.1^{b}	70.3^{b}	2.13	0.73^{b}	3.00^{b}
6	NC+ 1000 FTU/kg	0.456^{c}	1.461^{c}	1.226^{bc}	1.641^{bc}	1.494^{bc}	35.7^{c}	117.8^{b}	70.8^{b}	2.63	0.25^{b}	3.00^{b}
7	NC+ 2000 FTU/kg	0.487^{b}	1.600^{a}	1.218^{c}	1.599^{cd}	1.468^{de}	38.0^{a}	127.5^{a}	76.2^{a}	3.57	0.00^{b}	3.67^{b}
8	NC+ 3000/1000 FTU/kg	0.501^{a}	1.536^{b}	1.204^{c}	1.678^{b}	1.503^{bc}	38.7^{a}	124.2^{a}	75.2^{a}	2.86	0.24^{b}	3.25^{b}
	ANOVA											
	Pooled SEM	0.006	0.029	0.005	0.024	0.013	0.5	2.1	1.2	0.00	0.00	0.01
	Probability (P-value)	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.309	0.001	0.012
	REGRESSION											
	Linear[1]	<0.001	<0.001	0.011	<0.001	<0.001	<0.001	<0.001	<0.001	0.850	0.005	0.197
	Quadratic[2]	<0.001	<0.001	0.038	<0.001	<0.001	<0.001	<0.001	<0.001	0.696	0.004	0.037

[1]Linear effects of supplementing increasing levels of phytase

[2]Quadratic effects of supplementing increasing levels of phytase

Discussion

In the current study, broilers fed low-nPP diets exhibited lower nutrient and amino acid digestibility, reduced bone mineralization, as well as poorer growth performance compared to birds fed adequate nPP diets (Table V-3, V-4, and V-5). Reducing nPP levels in the NC negatively impacted energy (IDE) and amino acid digestibility compared to diets sufficient in nPP. Although not fully understood, it has been suggested that phytate elicits an adverse effect on energy and protein utilization through the formation of complexes, increases in endogenous losses, and the binding of energy-generating nutrients (lipids and carbohydrates) and endogenous enzymes (Cosgrove, 1980; Cowieson et al., 2006a; Selle and Ravindran, 2007; Selle et al., 2000; Thompson et al., 1987). Ravindran et al. (2006) reported that increasing dietary concentrations of phytate impaired energy utilization and ileal digestibility of protein and amino acids, suggesting that the anti-nutritive properties of phytate can hinder the availability of not only minerals, but energy and amino acids as well. Digestibility of energy and amino acids were increased in response to the addition of phytase to the low-nPP diet in the current study. Increasing concentrations of phytase resulted in a 2 to 5.7% increase on d 14 and a 6.8 to 10.4% increase in total amino acid response on d 28 with linear and/or quadratic improvements being detected during each phase. It was apparent that phytase inclusion had a greater impact on nutrient utilization as bird age increased, with energy and all amino acids being influenced at an older age compared to little impact on energy and a few amino acids (phenylalanine, methionine, arginine, and leucine) early in the study. Li et al. (2015) reported similar findings with improvements

in amino acid digestibility being more pronounced when including phytase in older (d 19 to 21) compared to younger birds (d 7 to 9). Additionally, in the current study, improvements in amino acids varied with greater responses being observed with tryptophan, threonine, and valine and a less pronounced increase in digestibility being detected with arginine, methionine, and histidine, when evaluating phytase inclusion on d 28. In a review of twenty-four published articles, Cowieson et al. (2017) noted higher responses with cysteine, threonine, serine, glycine, and valine and lower responses with arginine, glutamine, and methionine with phytase addition. It is possible that these improvements in response to phytase can be attributed to the hydrolysis of phytate complexes leading to increased liberation of proteins and amino acids for digestion and absorption (Onyango et al., 2004; Selle et al., 2000). An additional mechanism proposed by Cowieson et al. (2004) suggests that phytase may ameliorate the anti-nutritive effects of phytate, therefore, "sparing" amino acids through reductions in endogenous losses. It has been reported that phytase may modulate the secretion of mucin, which is relatively rich in threonine, serine, and proline, through the destruction of phytate, therefore limiting the excretion of mucin and endogenous amino acids (Cowieson et al., 2004; Cowieson and Ravindran, 2007; Onyango et al., 2008). Amerah et al. (2014) reported a strong correlation between amino acid digestibility and the degree of phytate degradation with the inclusion of phytase (1,000 FTU/kg) improving the average digestibility of all measured amino acids by 12.3% on d 21 (74.8 vs 84.0). Higher doses of phytase have been associated with the further degradation of phytate resulting in improved amino acid digestibility (Cowieson et al., 2009; Shirley and Edwards, 2003). In the present study,

supplementing super-doses of phytase (2,000 and 3,000/1,000 FTU/kg) further increased amino acid digestibility beyond that of 500 FTU/kg. Birds fed diets with the inclusion of super-doses (2,000 and 3,000/1,000 FTU/kg) yielded the highest amino acid digestibility amongst phytase treatments throughout the study. These further benefits in digestibility could potentially be due to the increased disruption of the phytate structure releasing bound nutrients and/or the reduction of endogenous amino acid secretions.

The impact of phytase on energy digestibility has previously been evaluated with consistent improvements in energy utilization being reported (Namkung and Leeson, 1999; Ravindran et al., 2000; Ravindran et al., 2001; Santos et al., 2008; Selle et al., 2007). Increasing doses of phytase in the present trial resulted in linear and quadratic increases in IDE with improvements ranging from 4.55 to 10%. Similarly, Shirley and Edwards (2003) reported linear increases ($P = 0.0001$) in energy utilization with increasing phytase concentrations up to 12,000 U/kg. Phytase supplementation in the present study tended to influence ($P = 0.063$) IDE during the earlier phase of the study with a greater impact ($P < 0.05$) being observed at the conclusion of the trial. Although the exact mode of action remains unknown, it has been speculated that energy improvements associated with phytase may be due to increases in protein absorption or greater digestibility of starch and lipid through the dissolution of the phytate-complexes (Camden et al., 2001). The ability to mitigate endogenous losses of nutrients through phytase supplementation may also increase metabolizable energy by reducing the energy required for maintenance, therefore, allowing a greater amount of energy for growth (Wu et al., 2015). Cowieson et al. (2009) concluded that depending on the amino acid

composition of the protein, the energy value of the diet may be directly affected by endogenous protein loss. The improvements in energy digestibility observed in the present study could potentially be a multi-faceted and complex combination of mechanisms involving the increase in the digestibility of amino acids. Therefore, improving energy utilization, and/or the liberation of energy generating components such as starch and lipids resulting in greater metabolizable energy values. Santos et al. (2008) attributed improvements in metabolizable energy (65 to 195 kcal/kg) to improvements in protein, starch and fat digestibility when evaluating increasing levels of phytase (500, 750, and 1,000 U/kg) in diets fed to birds through 21 days of age. Similarly, Ravindran et al. (2000) detected an increase in energy (96 to 120 kcal/kg) when supplementing phytase (400 or 800 FTU/kg) in diets with varying levels of phytic acid. The incorporation of super-doses of phytase in the current study resulted in improvements of 153 and 152 kcal/kg, respectively, in IDE beyond that of the 500 FTU/kg treatment. However, contrasting data published by Pirgozliev et al. (2008) and Cowieson et al. (2006b) indicated that higher doses of phytase (> 2,000 FTU/kg) had relatively little additional effect on energy utilization compared to lower doses (≥ 500 FTU/kg).

As expected, reducing the nPP content of the NC diet negatively impacted P digestibility throughout the study with a 32.7% (0.539 vs. 0.363) and a 19.4% (0.545 vs. 0.439) reduction being observed on d 14 and 28 respectively. Increasing supplemental doses of phytase linearly and quadratically ($P < 0.001$) increased ileal P digestibility throughout the trial. Numerous studies have exhibited improvements in P availability

when supplementing phytase in low-P broiler diets (Ahmad et al., 2000; Gautier et al., 2017; Ravindran et al., 2000; Silversides et al., 2004). Data published by Tamim et al. (2004) suggested that the supplementation of phytase increases the hydrolysis of dietary phytate resulting in improved P absorption. Furthermore, Shirley and Edwards (2003) reported linear (P = 0.0005) and quadratic (P = 0.0001) improvements in P retention (53.8 to 79.9%) as a result of greater phytate disappearance (49.5 to 94.8%) when increasing graded levels of phytase (375 to 12,000 U/kg) in low-P diets. In the present experiment, greater improvements in ileal P digestibility were observed as phytase concentration increased indicating that P digestibility may be attributed to the enhanced liberation of phytate bound P via phytase inclusion (Ravindran et al., 2006; Woyengo and Nyachoti, 2011). It is noteworthy that phytase at super-dose concentrations (2,000 and 3,000/1,000 FTU/kg) yielded the highest P digestibility amongst treatments throughout the study with further improvements of 32.8 and 17.3%, respectively, being detected compared to the 500 FTU/kg treatment at the end of the trial. These results are not surprising as it has been suggested that phytase at super-dose concentrations leads to greater destruction of the phytate structure, thus, releasing additional P molecules for absorption (Cowieson et al., 2011). Other authors detecting further increases in P digestibility beyond conventional doses have published similar results (Manangi and Coon, 2008; Manobhavan et al., 2015; Zhang et al., 2000).

The addition of phytase has been suggested to alleviate the binding of Ca to phytate, improve Ca solubility, and allow potential reductions in dietary Ca (Walk et al., 2012). While improvements in Ca digestibility have been published (Akter et al., 2018;

Bradbury et al., 2018; Ravindran et al., 2006), others have noted variable responses with phytase addition (Amerah et al., 2014; Hamdi et al., 2018). In the present study, it was interesting to note that the reduction in nPP in the NC diet increased ileal digestibility of Ca with increasing concentrations of phytase linearly and quadratically reduced Ca digestibility. Dilger et al. (2004) also observed an increase in ileal Ca digestibility when feeding male broilers reduced nPP diets. Data reported by Sommerfeld et al. (2018) indicated a greater disappearance of Ca in the ileum of birds fed diets low in dietary P compared to birds fed adequate P diets. Furthermore, Hamdi et al. (2018) noted a decrease in ileal Ca digestibility (59.26 vs. 64.52) when including phytase in diets with high and low Ca:avP ratios. It was speculated that the influx and efflux of Ca in the small intestine might be modulated by the increase of P absorption and growth response observed with phytase addition. In the current study, the lowest concentration of phytase (250 FTU/kg) yielded the highest d 14 Ca digestibility amongst phytase treatments with birds fed super-doses exhibiting the lowest digestibility. It is possible that the considerable rise in FC observed with increasing concentrations of phytase modulated the absorption of Ca in the small intestine, thus lowering the overall Ca digestibility. Pansu et al. (1981) suggested that greater consumption of dietary Ca in rats resulted in a down-regulation of Ca absorption while lower consumption up-regulated Ca absorption. As the birds aged, the inclusion of increasing concentrations of phytase up to super-dose levels had no additional influence on the ileal digestibility of Ca. Manobhavan et al. (2015) observed similar results with Ca digestibility remaining parallel amongst phytase treatments containing 500, 2,500, and 5,000 FTU/kg.

Tibia bone ash has been considered an ideal criterion for evaluating phytase efficacy on mineral utilization and deposition in broilers (Lalpanmawia et al., 2014; Tang et al., 2012). In the current trial, bone mineralization (ash, Ca, and P) was adversely affected in birds fed the low-nPP diet, which may have resulted from insufficient levels of P available for bone development. Lan et al. (2012) experienced similar results when feeding low-P diets (0.21% avP) to male broilers with the reduction in dietary P decreasing tibia bone ash, Ca and P content. Additionally, Panda et al. (2007) noted a decline in tibia ash concentration as dietary nPP levels were reduced in 21 day old broilers. In the present experiment, linear and quadratic improvements in bone mineralization were detected as phytase concentration increased. Data from Dilger et al. (2004) and Taheri et al. (2015) indicated linear and quadratic responses ($P < 0.05$) in a series of trials evaluating increasing doses of phytase on tibia ash. Rousseau et al. (2012) proposed that the addition of phytase in the current study improved the availability of P in the gastrointestinal tract resulting in greater digestibility of P and higher concentrations of substrate for bone mineral deposition, as observed in tibia ash weight and P content in the current study. Furthermore, phytase fed at super-dose concentrations yielded the highest tibia ash, P, and Ca content amongst treatments with further improvements in ash and P content beyond that of conventional doses due to the potential liberation of greater portions of minerals. Manobhavan et al. (2015) demonstrated super-dosing phytase (2,500 FTU/kg and 5,000 FTU/kg) increased tibia ash by 7.1 and 12.9%, improved tibia Ca content by 9.3 and 14.3%, and improved P content by 11.5 and 17.9%, respectively, compared to phytase concentrations at 500

FTU/kg. Additional work by Augspurger and Baker (2004) and Shirley and Edwards (2003) demonstrated the ability of super-dosing phytase (1,500 to 12,000 FTU/kg) to further enhance tibia ash concentrations compared to conventional doses.

Advantages in growth performance due to phytase inclusion in broiler diets have been extensively documented (dos Santos et al., 2014; Onyango et al., 2004; Powell et al., 2011; Ravindran et al., 2008; Selle and Ravindran, 2007; Singh, 2008). Throughout the current trial, birds fed diets deficient in nPP exhibited the lowest feed consumption, resulting in the lightest BW, poorest FCR, and highest mortality amongst dietary treatments. It is believed that the anti-nutritive properties related to phytate may suppress appetite and impede nutrient availability by adversely affecting FC and depressing growth performance (Cowieson et al., 2011; Selle and Ravindran, 2007). Previous studies with low-P concentrations reported reduced FC and poorer growth performance (Bradbury et al., 2014; Ceylan et al., 2012; Wu et al., 2004a) which are consistent with the findings described in the present paper. Additionally, birds fed diets deficient in P experienced higher mortality throughout the present study as a potential result of lower P levels in the diet. Waldroup (1999) suggested that failing to supply adequate amounts of P in the diet can severely hinder broiler performance and lead to excessive mortality. Vieira et al. (2015) reported greater mortality in low P diets (0.14 and 0.20 nPP) compared to diets with higher levels of dietary P. The addition of phytase in the low-nPP diet, regardless of dose, improved mortality to levels similar to that of the diet adequate in dietary nPP. Supplementing increasing levels of phytase linearly and quadratically ($P < 0.05$) improved all performance parameters (FC, BW, FCR, and mortality) measured

in the current experiment. The improvements in BW observed in the current study can be attributed to an assorted combination of increases in FC and nutrient availability as phytase concentrations are increased. These results are confirmed by work published by Santos et al. (2008) in which the performance improvement observed with phytase addition was a result of greater utilization of energy, protein, amino acids, and minerals. Additionally, Pirgozliev et al. (2011) credited increases in dietary energy, amino acid availability, and FC with improvements in growth performance from phytase inclusion.

The practice of super-dosing phytase has become an opportunity for producers with elevated doses (≥ 1500 FTU/kg) yielding further improvements in performance compared to conventional inclusion rates. In the current experiment, the implementation of a "step-down" treatment (3,000/1,000 FTU/kg) was aimed to determine if feeding higher concentrations in the starter and reducing dosage in subsequent phases is feasible in order to reduce feed costs without sacrificing performance. While the addition of phytase at 3,000 FTU/kg produced the highest digestibility, bone mineralization, and growth performance in the starter period, "stepping down" phytase concentrations to 1,000 FTU/kg in the treatment during the grower phase resulted in a less pronounced impact on all parameters evaluated compared to birds fed 2,000 FTU/kg throughout the study. Gehring et al. (2014) reached similar conclusions when evaluating the effects of a "step-down" phytase program on broiler performance. Similar to the present experiment, the authors reported a negative impact on broiler performance when experiencing a three-fold change in phytase levels throughout the trial. It was concluded that higher doses of phytase should be fed through to market age in order to maximize performance

response. Super-dosing phytase at 2,000 FTU/kg in the present study enhanced the digestibility of amino acids, energy, and minerals, in addition to a further response in FC resulting in heavier BW and improved feed efficiency compared to birds fed lower phytase doses. As stated by Cowieson et al. (2011), the "extra-phosphoric" benefits associated with higher levels of phytase supplementation may be attributed to the further destruction of the phytate structure, resulting in greater alleviation of the antinutritive properties and the potential generation of more soluble phytate esters and myo-inositol, therefore, translating to enhanced nutrient digestibility, greater bone mineralization, and improved broiler performance.

In the present study, improvements in nutrient digestibility, bone mineralization, and broiler performance were observed with increasing doses of phytase in low-nPP diets. Furthermore, the inclusion of super-doses of phytase yielded a more pronounced effect on all parameters measured compared to phytase supplemented at conventional doses demonstrating that elevated levels of phytase can result in further improvements in performance related to increases in FC and nutrient digestibility.

CHAPTER VI

CONCLUSION

The inclusion of exogenous enzymes in broiler diets has become an important strategy for poultry producers looking to reduce feed costs without sacrificing bird performance. Incorporating exogenous enzymes in poultry diets enables the use of higher levels of lower-quality ingredients, greater formulation flexibility and reduced variation between ingredient batches. Furthermore, the supplementation of exogenous enzymes can effectively enhance the absorption and utilization of nutrients that are typically inaccessible by monogastric animals translating to greater efficiency and improved growth performance. Although the use of exogenous enzymes in poultry diets has been practiced for the past several decades, the multi-faceted effect of rising raw material costs, greater availability of alternative and by-product ingredients, as well as lower enzyme costs has warranted further research regarding the use of exogenous enzymes as a feeding strategy.

When evaluating the impact of a cocktail NSPase in diets manufactured with low and high-quality corn (Chapter I), the incorporation of corn-screenings negatively impacted performance early on in the study with birds exhibiting higher FCR and lighter BW. Furthermore, reducing the energy content of the diet negatively influenced FCR throughout the study compared to diets sufficient in dietary energy. The addition of NSPase enhanced FCR throughout the study and elicited a greater response in diets with corn screenings compared to diets absent of screenings. These data indicate that the

addition of cocktail NSPase allows for the incorporation of lower-quality ingredients in order to reduce feed costs without negatively impacting broiler performance.

In Chapter II, a series of trials were conducted to determine the effect of a cocktail NSPase in low-energy diets when fed to male broilers. As expected, the 132 kcal/kg reduction in energy resulted in lighter BW and poorer FCR in each study compared to birds fed the positive control (PC) diet. These negative impacts on performance parameters observed when decreasing the caloric value of the diet translated to a reduction in carcass weight and yield (experiment 1 and 2). In experiment 1, the addition of NSPase improved FCR with a 2 point reduction being observed in the starter period. The supplementation of NSPase in experiment 2 yielded consistent increases in BW throughout the study and reduced FCR to levels comparable to the PC. These improvements in live performance correlated to a positive impact on processing parameters with higher WOG weights and yield being observed compared to the negative control (NC). Similar results were observed in experiment 3, with the inclusion of NSPase increasing BW to levels similar to that of the PC diet throughout the trial and reducing starter FCR. These trials demonstrate the ability to effectively remove energy from the diet when supplementing NSPase in order to reduce diet costs without hindering bird performance or processing parameters.

Evaluating the impact of increasing phytase levels on nutrient digestibility, bone mineralization, and growth performance in low-phosphorus diets (Chapter III) demonstrated the potential use of higher doses of phytase to further enhance nutrient utilization and broiler performance. When reducing dietary nPP levels, nutrient

digestibility and bone mineralization decreased leading to poorer growth performance. The inclusion of increasing concentrations of phytase linearly and quadratically improved amino acid and nutrient digestibility (IDE, Ca, P), bone mineralization (ash percent, Ca and P), and broiler performance (BW, FC, FCR). Furthermore, the addition of phytase at super-dose concentrations elicited a further impact on digestibility, mineralization, and growth performance compared to birds fed phytase at conventional levels illustrating the additional benefits associated with higher phytase levels.

The results of the current experiments demonstrate the potential cost savings and performance advantages that may be achieved with exogenous enzyme addition. The ability to supplement exogenous enzymes enables producers to successfully incorporate lower quality ingredients, modify nutrient density, and enhance nutrient digestibility leading to greater performance benefits, lower diet costs, and higher overall profit margins.

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